Analysis of Minerals (Na, K, Ca and Fe) in dairy products Using Atomic Absorption Spectroscopy (AAS)

By
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DECLARATION

This research project described in this thesis was carried out by my self at SGS Lanka (Pvt.) Ltd. under the supervision of Mrs.A.S.Perera. Lecturer, Department of Food Science and Technology and Mr.H.A.P.Indrajith, Head-Natural Resources Laboratory, SGS Lanka (Pvt) Ltd. during the industrial training from 26th March to 26th August and this is not a copy of any other theses published earlier.

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AFFECTIONATELY DEDICTED TO MY LOVING PARENTS AND TEACHERS

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ABSTRACT

Determination of major minerals Like Na, K, Ca and Fe like trace minerals is a common practice of quality control laboratories of dairy industry. Because some dairy products manufacturers advocate their products as fortified with above minerals, but the actual composition of these mineral is often not indicated on the product labels. Analysis of above minerals by Flame Atomic Absorption Spectroscopy (FAAS) appears to be quite straight forward.

First approach was to develop a method to analyze four minerals in milk powders available in the market. The suggested method is a combination of an in house method and AOAC method which is a derivative of AOAC method. The accuracy of this method highly depends on sample preparation. The organic fraction must be removed from the mineral to avoid interference in the signal readout. The suggested method resulted the recovery percentages of 89%, 65% and 85% for Na, K and Fe respectively. According to the Mann Whitney statistical approach the method is more accurate for determination of Na in milk powders as the results have shown very close values for three milk powder categories out of five.

The second approach was to analyze mineral composition in other dairy products. For that purpose an in house method was used to analyze the four mineral levels in dairy products available in the market. Replicates were performed to get the most accurate value. Other dairy products such as Yoghurt, Curd, Ice cream, Butter, Butter Oil and Margarine products were analyzed based on the selected categories available in the market. Liquid milk products were directly analyzed without separating the organic fraction .It may affect for the accuracy of the test due to the interaction of organic fraction. Other dairy products were analyzed by separating the organic fraction by ashing. Kruskal Wallis statistical approach was applied to compare the results obtained for other dairy products. From the results it can conclude that there is no any significant difference among the brands of selected categories. By that can predict that the Na%, K%, Ca% and Fe% levels were approximately same for Curd, Yoghurt and Ice cream varieties most available in the local market.

A product wise comparison based on the analyzed mineral fraction was performed using Kruskal Wallis statistical approach. The results concluded that there is no any significant difference among Curd, Yoghurt and Ice cream based on the tested minerals. Each product does not give an added nutritional benefit in the form of tested minerals.

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ABBREVIATIONS

AOAC	official methods of Analysis of the Association of Official Analytical Chemistry
°C	Celsius
eg:	example
et al	and others
°F	Farenheights
gcm^{-3}	grams per cubic centimeter
$gmol^{-1}$	grams per mole
HNO_3	Nitric acid
$La(NO_3)_3$	Lanthanum nitrate
l	liter
mg	milligrams
ml	milliliter
M	Molarities
Na+	Sodium ion
N_2O	Nitrous oxide gas
PO_4^-	Phosphate ion
ppm	parts per million
T^o	Temperature
TS	Total Solids

CHAPTER 01

01 Introduction

Mineral content is a measure of quantitative specific inorganic components present in a food material. Minerals are an essential group of nutrients require for human body for optimal function. And also their presence is important for nutritional reasons, quality aspects and microbiological stability.

Milk and milk products are rich in minerals; especially in calcium. It is a common practice of determination of concentration of such minerals in their products in quality control laboratories of the dairy industry and the manufacturers of infant formula. (Danielson et al, 1988)

Different foods contain different amounts of minerals. Eating a varied diet will help ensure an adequate supply of most minerals for healthy people. In the UK, iron and calcium intakes are gradually decreasing. Most people don't show signs of deficiency but this does not mean their intake is ideal. Some adolescent girls and women of childbearing age may be deficient in iron, and at risk of iron deficiency anemia. There is also concern about the calcium intake of some adolescent girls and young women. (Henderson et al, 2003)

Additionally some dairy companies have embarked on advertisement campaigns concerning the nutritional quality of their products. In many cases the manufacturers of instant milk powders advocate their products as having been fortified with several mineral elements such as Na, K, Ca and Fe. However the actual composition of individual mineral element is often not indicated on the product labels of these milk powders (Akpanyung, 2006)

Therefore the determination of these four metal ions in milk products are often carried out by Atomic Absorption Spectroscopy(AAS).(Danielson et al, 1988).Determination of Na, K ,Ca and Fe in dairy products by Flame Atomic Spectroscopy(FAS) appears to be straight forward , but it's accuracy is depended on sample preparation.(Carazo et al.,1997)

SGS Lanka Pvt Ltd is clear global leader and innovator in inspection, verification, testing and certification services. So it needs to develop a method to analyze above mentioned minerals in dairy products.

Objectives:

General Objective:

- To give a comparative account on the mineral contents (Na, K, Ca and Fe) in different milk products available in the market.

Specific Objectives:

- To develop a method to analyze minerals (Na, K, Ca and Fe) in dairy products

- To determine the accuracy of the mineral contents given in the table.

CHAPTER 02

02 Literature Review

2.1 Milk

Milk is the fluid secreted by all mammalian species. And this milk is one of the few foods stuffs consumed in its natural state and it is the main raw material used for all milk products. (Lampert, 1987)

MILK PRODUCTION

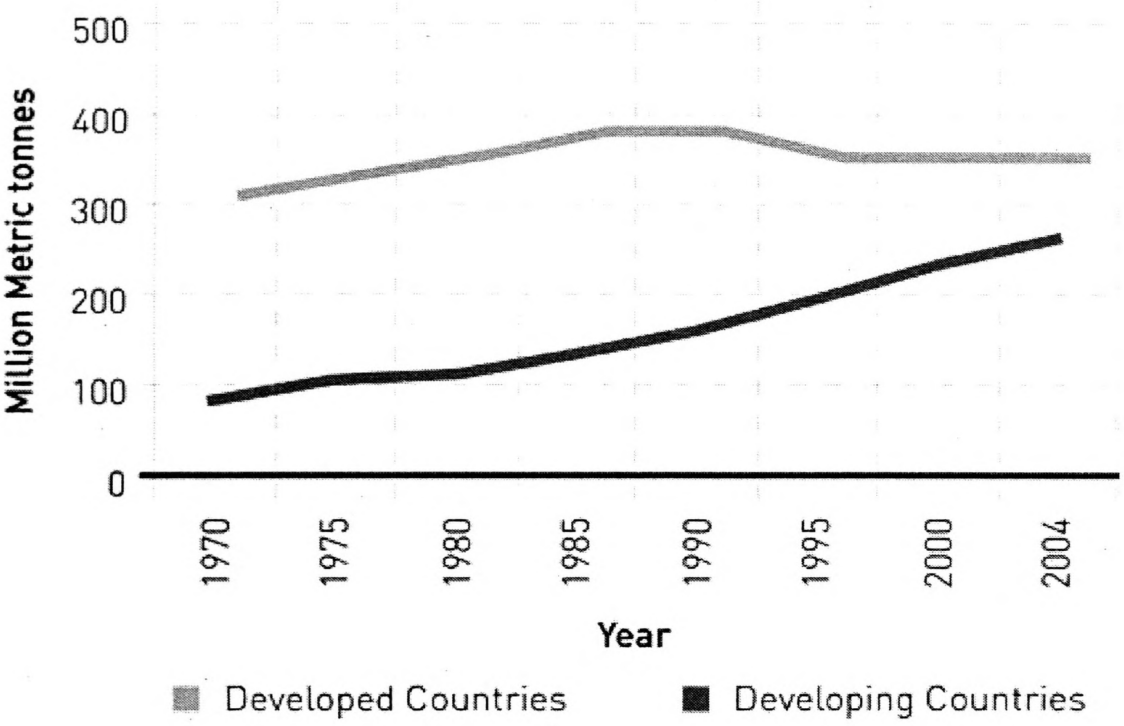

Figure 2.1 Annual milk productions in developed and developing countries
(Source: FAO, 2005)

Milk is a major food stuff that contains most of the nutrients need for human nutrition. But some minerals such as Iron, Copper and Manganese and also some vitamins are not present in sufficient quantities or in the proper proportions to apply the requirements for complete nutrition. (Lampert, 1987)

Milk has been defined as an emulsion of fat globules in a suspension of casein micelles (composed of casein, calcium and Phosphorous) all suspended in an aqueous phase which contains solubilized lactose, whey proteins and mineral salts. (Akpanyung, 2006) Milk has an excellent nutrient profile providing significant amounts of high quality proteins, Calcium, Sodium like minerals and vitamins. Drinking milk may help to reduce the risk of kidney

stones. A recent epidemiological study of more than 81,000 women with no history of kidney stones links intake of nonfat milk with decreased risk of colon cancer. As well as milk intake may help to reduce the risk of tooth decay by acting as a substitute for saliva.

2.2 Milk products

Milk is the main raw material for all milk products. Both milk and milk products play an important role in human nutrition. Fresh cow milk is reported to contain about 88% of water. During processing the water content of the milk is reduced to a very low level. (Akpanyung, 2006)

- Raw milk
- Dried milk products
  - 1.Dried whole milk
  - 2.Dried non fat
- Butter (salted)
- Butter oil (GHEE)
- Sterilized milk
- UHT treated milk
- Cheese
- Ice cream
- Margarine
- Fermented milk products
  - 1.Curd
  - 2. Yoghurt
  - 3. Kefir
  - 4.Kumiss
  - 5.Acidophilus milk

2.2.1 Dried milk products / Milk powder

Dried milk products are the products resulting from the removal of water from milk and milk products. The principle dried milk products are dried whole milk, dried skim milk, dried butter milk, dried whey and dried cream. These products are also known as whole milk powder, skim milk powder, butter milk powder, powdered cream or cream powder and whey powder. (Jacobs, 1999)

Definitions

- **Whole milk powder, dried whole milk:**

It shall be the material prepared by spray drying or roller drying of cow or buffalo milk. Whole milk is which has been standardized to the requirements for fat as 26.0% minimum.

- **Skimmed milk powder, dried skimmed milk:**

This is prepared by spray drying or roller drying of skimmed milk of cow or buffalo or a mixture there of. (SLS 731:1986)

Spray dried milk is produced by atomizing a spray of hot precondensed milk (TS 40 – 50%) inside a large chamber where it meets hot air entering at up to 190°C (375°F) foe whole milk and up to 260°C (500°F) for skimmed milk. (Ramasamy, 2000) Dried milk products are

generated when the water content has been reduced to less than 4 %.(Akpanyung, 2006). The milk dries instantaneously and the falling powder is quickly removed. Roller dried milk is produced by running or spraying pre condensed milk (up to 24% TS) .The milk dries almost instantaneously on the roller and the powder is removed by scrapers and rapidly cooled. (Ramasamy, 2000)

2.2.1 Butter

Butter is the product obtained by churning the cream which has been separated from warm milk. It is then "worked", i.e. excess water is removed. When cream is churned the fat droplets coalesce and form progressively larger clusters of fat globules. These grains finally break away from the surrounding liquid and form a semi-solid or plastic material called butter. (Jacobs, 1999).Then the mass is rendered homogeneous usually with the addition of salt and with or without addition of coloring matter. (Ramasamy, 2000)

In the U.K. butter tends to be prepared from comparatively fresh milk, but in many commonwealth countries the higher acidity of the cream used is often first partially neutralized with bicarbonates. (Ramasamy, 2000)

2.2.2 Butter oil

Butter oil is the product made by clarifying butter by means of melting and centrifuging. It contains practically no water or any of the other components of butter and consequently is almost entirely butterfat. (Jacobs, 1999) It shall be semi solid at 30°C and be creamy white in color.

Definition:

A pure clarified milk fat exclusively derived from the milk of the cow or buffalo or any mixture there of with out any foreign fat or oil and not containing any foreign substances. (SLS 340:1975)

2.2.3 Sterilized milk

The milk that has been heated without appreciable loss of volume to a T° of 100°C for a length of time sufficient to kill all organisms present and contained for delivery in hermetically sealed containers. (SLS 181:1983)

2.2.4 UHT treated milk

The milk that has been heated without appreciable loss of volume to a T° of 132°C – 200°C for not less than one second and shall then be filled and sealed aseptically into sterile containers in which it is to be supplied to the consumer.(SLS181:1983)

2.2.5 Cheese

Cheese is the food product made from the separated curd obtained by coagulating the major milk protein of whole milk, skimmed milk or milk enriched with cream.
Cheese shall be of the following four types:

- Hard cheese
- Semi-hard cheese
- Soft cheese
- Processed cheese or cheese spread

2.2.6 Ice cream

Definition:

A frozen sweetened product made from a heat treated mix consisting of edible fat and milk solids with or without other ingredients and permitted additives. (SLS 223:1989)

The product is made from a combination of milk products and two or more of the following ingredients; eggs, water and sugar with harmless flavoring and coloring with or without stabilizer and in the manufacture of which freezing is accompanied by agitation of ingredients (Jacobs, 1999).The product is intended for storage, sale and consumption in the frozen state. (SLS 223:1989)

Categories:

Ice cream can be categorized into three categories.

1. Simple Ice cream: A sweetened product made from a mix consisting of edible fat and milk solids with color, flavor, emulsifier and stabilizer.
2. Complex Ice cream: Simple ice cream with any one or more of the optional ingredients.

e.g.: fruits and fruit products

Nuts free from damages, rancidity and infestations

Food ingredients intended to impart flavor, taste or texture (e.g.; cocoa, chocolate, coffee, honey and treacle etc.)

3. Novelties: Single serve packs of either simple or complex ice cream with an outer edible coating such as chocolate, nuts, biscuits etc. (SLS 223:1989)

2.2.7 Margarine

Margarine can be described as a liquid emulsion or a food in the plastic form. It must contain no less than 80% fat as determined by the "Official Method", used the by the "Official Analytical Chemists".

2.2.8 Fermented milk products

Fermented milk is a product obtained from milk by the lactic fermentation through the action of single or mixed strains of lactic acid bacteria or by lactic fermentation accompanied by alcoholic fermentation by yeast with or without permitted additives.(SLS 824:part 1:1988)

Fermented milk was used by the people of Eastern Europe and minor long before the discovery of bacteria. (Lampert, 1987).Milk is fermented for the purpose of preservation. And this fermentation process accompanied by microorganisms. A number of organisms have been used to ferment milk one of the most common is *lactobacillus bulgaricus*. Butter milk, Kefir, Koumiss, Curd, Yoghurt and acidophilus milk have been categorized under fermented milk products. These products do not include milk coagulated by the addition of acids, milk coagulating enzymes etc.

2.2.8.1 Curd

Fermented milk product obtained from coagulation of cow or buffalo milk or a mixture thereof by the agency of following species of organisms.
Streptococcus lactis, streptococcus diacetylactis, streptococcus cremoris singly or in combination with *leuconostoc spp.,lactobacillus bulgaricus* and *streptococcus thermophyllus* Curd can be firm solid and free of lumps. (SLS 824: part 1:1988)

2.2.8.2 Yoghurt

Yoghurt is one of the most popular fermented milk products and has gained widespread consumer acceptance as a healthy food. (Mackinley, 2005).It is known by

different names according to the place where it is made; for e.g.: in America it is known as Matzoon; Leben in Egypt; Gioddu in Italy and Dadhiin in India. (Lampert, 1989)

Yoghurt provides an array of nutrients in significant amounts in relation to its energy and fat content, making it a nutrient dense food. In addition Yoghurt can provide the body with significant amount of Ca in a bioavailable form Furthermore yoghurt has many health benefits beyond the basic nutrition it provides such as improved lactose tolerance, a possible role in body weight and fat loss and a variety of health attributes associated with probiotic bacteria. (Mackinley, 2005)

Yoghurt shall be of the following three types.

1. Yoghurt : product with a fat content of 3.0% and above and not containing any added fruit or flavoring essences
2. Low fat Yoghurt : product with a fat content of less than 3.0% but more than 0.5% and not containing any added fruit or flavoring essences.
3. Non fat Yoghurt : product with a fat content of less than 0.5% and not containing any added fruit or flavoring essences (SLS 824:part 2 :1988)

2.3 Minerals in dairy products

2.3.1 Importance of minerals

The mineral elements are an important group of nutrients required by the human body for optimal functions. They can be divided into macro minerals (major elements) and micro minerals (trace elements). The macro minerals such as Sodium, Potassium, Calcium, Magnesium and Phosphorous are required by the body in amounts greater than 100mg per day where as the micro minerals such as Iron, Copper, Zinc and Manganese are required in amounts less than 100mg per day. (Akpanyung, 2006)

The mineral content of milk is fairly constant and is influenced but little by the feed.(Lampert, 1987) .For e.g. Iodine in milk is affected by feed supplements of iodine, the use of iodophor sanitizing solutions by the dairy industry, and the use of the iodine contained red food dye, erythrosine.

2.3.2 Physical and Chemical properties of selected metals

Table 2.1 physical and chemical properties of selected metals

Metal	Atomic number	Atomic mass $g\ mol^{-1}$	Density gcm^{-3}	Melting point °C	Boiling point °C
Sodium	11	22.9897	0.968	97.72	883
Potassium	19	39.0980	0.89	63.38	759
Calcium	20	40.0784	1.55	842	1484
Iron	26	55.8452	7.86	1538	2861

2.4 Health benefit and other properties of selected metals

2.4.1 Sodium

2.4.1.1 Introduction

Owing to its high reactivity, sodium is found in nature only as a compound and never as the free element. It is an essential element for animal life. As such, it is classified as a "dietary inorganic macro-mineral."

2.4.1.2 Recommended Dietary Allowances(RDA) for Sodium

1200 – 1500mg per day (McGee, 2007)

2.4.1.3 Factors affecting Na absorption

Sodium absorption occurs by different mechanisms in different parts of the intestine In the jejunum, sodium is mostly absorbed via co transport, as a result of active uptake of sugars and amino acids (both of these processes require the presence of Na^+).And in the ileum Na^+ itself is absorbed actively, against a significant electrochemical gradient. In the jejunum, sodium transport is greatly influenced by fluid movement and is stimulated by the presence of sugars but in the ileum, none of these factors affect sodium movement. Sodium is also actively absorbed in the colon. (Ellert,-1998)

2.4.1.4 Role in human health and disease prevention

Ionic form of Sodium is necessary for regulation of blood and body fluids, transmission of nerve impulses, heart activity, and certain metabolic functions.It is the main component of the body's extra-cellular fluids, and it helps carry nutrients into the cells.

Sodium also helps regulate other body functions, such as blood pressure and fluid volume, and works on the lining of blood vessels to keep the pressure balance normal. (Barron, 2006)

2.4.1.5 Some dairy products rich in Na

Gouda and cottage cheeses

2.4.1.6 Health problems related to less availability

People who consume less Na are at risk for hypoatremia characterized by lethargy, confusion, muscle twitching, seizures and coma.

2.4.1.7 Health risk of Na beyond threshold level

High sodium intake results in increased loss of calcium in the urine, possibly due to competition between sodium and calcium for reabsorption in the kidney or by an effect of sodium on parathyroid hormone (PTH) secretion. Each 2.3-gram increment of sodium excreted by the kidney has been found to draw about 24-40 milligrams of calcium into the urine. Because urinary losses account for about half of the difference in calcium retention among individuals, dietary sodium has a large potential to influence bone loss. In adult women, each extra gram of sodium consumed per day is projected to produce an additional rate of bone loss of 1% per year if all of the calcium loss comes from the skeleton. (Higdon, 2003)

2.4.2 Potassium

2.4.2.1 Introduction

Potassium is an essential dietary mineral that is also known as an electrolyte. The normal functioning of our bodies depends on the tight regulation of potassium concentrations both inside and outside of cells.

2.4.2.2 Recommended Dietary Intake (RDA) for Potassium

- 1950-5460 mg/day (for adults)

2.4.2.3 Role in human health and disease prevention

Potassium helps to reduce the amount of sodium in the body, and it is understood that high levels of sodium contribute to risk of stroke. (Turner, 1998) A limited number of enzymes require the presence of potassium for their activity. The activation of sodium, potassium-ATPase requires the presence of sodium and potassium. The presence of potassium

is also required for the activity of pyruvate kinase, an important enzyme in carbohydrate metabolism.Studies have shown that Potassium helps to prevent some chronic diseases such as Stroke,Osteoporosis and Kidney stones.(Higdon,2003)

2.4.2.4 Health problems related to less availability

Potassium deficiency can result in high blood pressure, stroke, congestive heart failure, cardiac arrhythmias, weakness, depression and glucose intolerance, as well as increased risk of kidney stones, and increased bone turnover. (National Dairy Council, 2007)

2.4.3 Calcium

2.4.3.1 Introduction

Calcium, the most abundant mineral in the human body, has several important functions. More than 99% of total body calcium is stored in the bones and teeth where it functions to support their structure. The remaining 1% is found throughout the body in blood, muscle, and the fluid between cells. Calcium is needed for muscle contraction, blood vessel contraction and expansion, the secretion of hormones and enzymes, and sending messages through the nervous system A constant level of calcium is maintained in body fluid and tissues so that these vital body processes function efficiently.

4.4.3.2 Recommended Dietary Allowances(RDA) for Calcium

Table 2.2 RDA values for Ca for different age groups.

Male Female age group	RDA value (mg/day)
Infants	**210-270**
Children	**500-1300**
Adults	**1000-1500**

(Source: National Institutes of Health, USA, 2005)

2.4.3.3 Factors affecting Ca absorption and excretion

Calcium absorption refers to the amount of calcium that is absorbed from the digestive tract into our body's circulation. Calcium absorption can be affected by the calcium status of the body, vitamin D status, age, pregnancy and plant substances in the diet. The amount of calcium consumed at one time such as in a meal can also affect absorption. For example, the efficiency of calcium absorption decreases as the amount of calcium consumed at a meal increases. (National Institutes of Health, USA, 2005)

2.4.3.4 Role in human health and disease prevention

Calcium helps to prevent more diseases such as osteoporosis and osteopenia. As well as research studies have shown that people who consume diets rich in Ca and low in fats have very less risk for blood pressure. Some studies suggest that increased intakes of dietary and supplemental calcium are associated with a decreased risk of colon cancer too. (National Institutes of Health, USA, 2005)

2.4.3.5 Some dairy products rich in Ca

Milk, Yoghurt and Cheese

2.4.3.6 Health problems related to less availability

Inadequate calcium intake, decreased calcium absorption, and increased calcium loss in urine can decrease total calcium in the body, with the potential of producing osteoporosis and the other consequences of chronically low calcium intake. Simple dietary calcium deficiency produces no signs at all. Hypocalcaemia can cause numbness and tingling in fingers, muscle cramps, convulsions, lethargy, poor appetite, and mental confusion .It can also result in abnormal heart rhythms and even death. (National Institutes of Health, USA, 2005)

2.4.3.7 Health risk due to high Ca

Adverse conditions associated with high calcium intakes are hypercalcaemia (elevated levels of calcium in the blood), impaired kidney function and decreased absorption of other minerals. Another concern with high calcium intakes is the potential for calcium to interfere with the absorption of other minerals, iron, zinc, magnesium, and phosphorus.

2.4.4 Iron

2.4.4.1 Introduction

Iron is the sixth most common element in the world and the fourth most abundant on earth. It is essential to most life forms and to normal human physiology. Iron is an integral part of many proteins and enzymes that maintain good health. Almost two-thirds of iron in the body is found in hemoglobin, the protein in red blood cells that carries oxygen to tissues. Smaller amounts of iron are found in myoglobin, a protein that helps supply oxygen to muscle, and in enzymes that assist biochemical reactions. Iron is also found in proteins that store iron for future needs and that transport iron in blood. Iron stores are regulated by intestinal iron absorption. (National Institutes of Health, USA, 2005)

2.4.4.2 Recommended Dietary Allowances (RDA) for Iron

Table 2.3 RDA values of Fe for different age group

Group	Male (mg/day)	Female (mg/day)
Infants	**11**	**11**
Children	**7 - 11**	**7 - 15**
Adults	**8**	**18**
Adults (> 50 yrs.)	**8**	**8**

(**Source:** National Institutes of Health, USA, 2005)

2.4.4.2 Factors affecting Fe absorption

Storage levels of iron have the greatest influence on iron absorption. Iron absorption increases when body stores are low. When iron stores are high, absorption decreases to help protect against toxic effects of iron overload. Iron absorption is also influenced by the type of dietary iron consumed. Absorption of heme iron from meat proteins is efficient. Absorption of heme iron ranges from 15% to 35%, and is not significantly affected by diet. In contrast, 2% to 20% of nonheme iron in plant foods such as rice, maize, black beans, soybeans and wheat is absorbed. Nonheme iron absorption is significantly influenced by various food components, such as meat proteins and vitamin C will improve the absorption of nonheme iron, but Tannins, calcium, polyphenols, and phytates decreases it. (National Institutes of Health, USA, 2005)

2.4.4.3 Role in human health and disease prevention

In humans, iron is an essential component of proteins involved in oxygen transport .It is also essential for the regulation of cell growth and differentiation. (National Institutes of Health, USA, 2005)

2.4.4.4 Some dairy products rich in Fe

Usually dairy products are poor sources of Fe. However they are fortified with Fe according to the customer requirement. e.g.: milk powders

2.4.4.6 Health problems related to less availability

A deficiency of iron limits oxygen delivery to cells, resulting in fatigue, poor work performance, and decreased immunity. Iron deficiency anemia can be associated with low dietary intake of iron, inadequate absorption of iron, or excessive blood loss. Women of childbearing age, pregnant women, preterm and low birth weight infants, older infants and

toddlers, and teenage girls are at greatest risk of developing iron deficiency anemia because they have the greatest need for iron .Women with heavy menstrual losses can lose a significant amount of iron and are at considerable risk for iron deficiency. (National Institutes of Health, USA, 2005)

2.4.4. Health risk due to high Fe

Excess amounts of iron can result in toxicity and even death. Iron can accumulate in body tissues and organs when normal storage sites are full. For example, people with hemachromatosis are at risk of developing iron toxicity because of their high iron stores. (National Institutes of Health, USA, 2005)

2.5 Atomic Spectroscopy

Atomic Spectroscopy owes it's origin to the work of Kirchoff and Bunsen in the 1850's who observed that atoms in flames absorb or emit radiation that is characteristic of that element. Over the years the Spectrometry has developed to such an extent, at present atomic absorption spectrometry or atomic emission spectrometry is used for quantitative and qualitative determination of about 70 elements in the periodic table excluding the elements of upper right and extreme right of the periodic table. (Pathiratne, 2004)

These two techniques are applicable to a large number of metals and metalloids in all matrices including food stuffs, ground water, industrial wastes and sludge...etc. Atomic spectroscopy is used to provide information about the type and concentration of minerals in foods. The type of minerals is determined by measuring the position of the peaks in the emission or absorption spectra. The concentration of mineral components is determined by measuring the intensity of a spectral line known to correspond to the particular element of interest.

Absorption Vs Emission

Both atomic absorption and atomic emission results due to the movements of atoms in between "ground state" and "excited state". Every element has a specific number of electrons associated with its nucleus. The normal and most stable orbital configuration of an atom is known as the "ground state." If energy is applied to an atom, the energy will be absorbed by the atom and an outer electron will be promoted to a less stable configuration known as the "excited state.". The energy that is absorbed by the atom is known as "Absorption energy". Since this state is unstable, the atom will immediately return to the

"ground state," releasing light energy. This energy represents the "Emission energy". (Perkin-Elmer corporation, 1996)

Atomic Absorption

The "ground state" atom absorbs light energy of a specific wavelength as it enters the "excited state." As the number of atoms in the light path increases, the amount of light absorbed also increases. By measuring the amount of light absorbed, a quantitative determination of the amount of analyte can be made. The use of special light sources and careful selection of wavelengths allow the specific determination of individual elements. (Perkin-Elmer corporation, 1996)

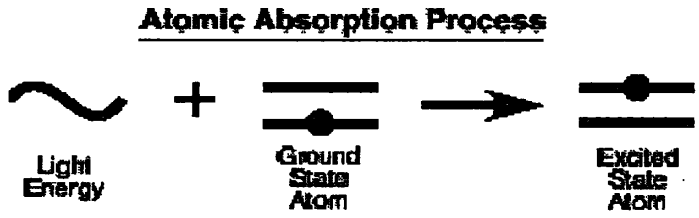

Figure 2.2 The process of Atomic Absorption
(Source: Perkin-Elmer corporation, 1996)

Atomic Emission

Simply the Emission is the opposition of Absorption However, since the excited-state is unstable; the atoms spontaneously return to the "ground state" and emit light. Therefore the analyte is subjected to a high-energy thermal environment in order to produce excited-state atoms. This environment can be provided by a flame or, more recently, a plasma. (Perkin-Elmer corporation, 1996)

The emission spectrum of an element consists of a collection of emission wavelengths called emission lines because of the discrete nature of the emitted wavelengths. The intensity at an emission line will increase as the number of excited atoms of the element increases. In atomic emission processes of both excitation and de-excitation or decay are involved.

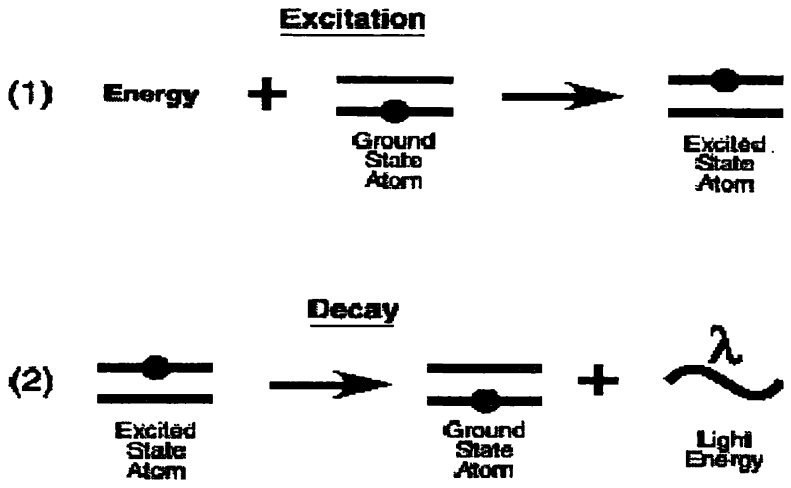

Figure2.3 The process of Atomic Emission
(Source: Perkin-Elmer corporation, 1996)

2.5.1 Atomic Absorption Spectroscopy

Atomic absorption spectroscopy (AAS) is an analytical method that is based on the absorption of UV-visible radiation by free atoms in the gaseous state. The food sample to be analyzed is normally ashed and then dissolved in an aqueous solution. This solution is placed in the instrument where it is heated to vaporize and atomize the minerals. A beam of radiation is passed through the atomized sample, and the absorption of radiation is measured at specific wavelengths corresponding to the mineral of interest. Information about the type and concentration of minerals present is obtained by measuring the location and intensity of the peaks in the absorption spectra. (McClements, 2003)

2.5.2 Atomic Emission Spectroscopy

Atomic emission spectroscopy (AES) is different from AAS, because it utilizes the emission of radiation by a sample, rather than the absorption. For this reason samples usually have to be heated to a higher temperature so that a greater proportion of the atoms are in an excited state (although care must be taken to ensure that ionization does not occur because the spectrum from ionized atoms is different from that of non-ionized atoms). There are a number of ways that the energy can be supplied to a sample, including heat, light, electricity and radio waves. (McClements, 2003)

2.5.3 Principles of Atomic Spectroscopy

The primary cause of absorption and emission of radiation in atomic spectroscopy is electronic transitions of outer shell electrons. The energy change

associated with a transition between two energy levels is related to the wavelength of the absorbed radiation.

$$\Delta E = hc/\lambda,$$

Where, h = Planks constant

c = the speed of light and

λ = the wavelength.

Therefore for a given transition between two energy states radiation of a discrete wavelength is either absorbed or emitted. Each element has a unique electronic structure and therefore it has a unique set of energy levels. Consequently, it absorbs or emits radiation at specific wavelengths. Each spectrum is therefore like a "fingerprint" that can be used to identify a particular element. In addition, because the absorption and emission of radiation occurs at different wavelengths for different types of atom, one element can be distinguished from others by making measurements at a wavelength where it absorbs or emits radiation, but the other elements do not. . (McClements, 2003)

For both atomic absorption and atomic emission analysis, the analyte must be converted into free atomic state. Many elements of interest do not exist in atomic form at ambient temperatures. A higher temperature environment near or above 2000 K therefore is needed to convert analyte into vapors. In general flames, furnaces, plasmas, electric arc and electric dischargers are used to produce a higher temperature environment for converting analyte into atomic vapours.Analyte introduced into these high temperature media volatilized, decomposed and dissociated forming atoms and ions. These atoms and ions are constantly colliding with each other during their random motions in the vapour phase. These collisions can excite outer shell electrons of atoms to higher electronic energy states. (Pathiratne, 2004)

2.5.3.1 Principle of Atomic Absorption Spectroscopy

In this approach since atoms absorb light at very specific wavelengths, it is necessary to use a narrow-line source which emits the narrow-line spectra of the element of interest. Narrow-line sources provide high intensity.(Perkin-Elmer corporation,1996).Usually the monochromator allows the isolation of the resonance line from other lines produced in the spectrum of the lamp, while blocking their path way. So this monochromatic radiation with a known intensity absorbable by analyte atoms in the atom cell is passed through the atomic vapor portion of the atomic vapour is absorbed by the analyte atoms and the remainder is transmitted out of the atom cell is shown below.

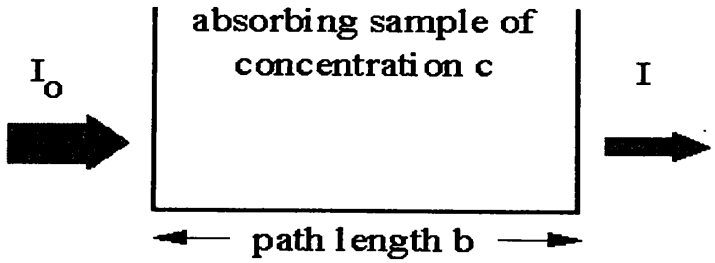

Figure 2.4 Absorption of resonance radiation by analyte atoms in the vapour phase (Source: Tissue, 1996)

Where,

I_0 = Intensity of incident monochromatic radiation with wavelength lamda

I = Intensity of transmitted monochromatic radiation with wavelength lamda

c = Concentration of analyte atoms in the vapor phase

ε = Absorption coefficient of analyte atoms at wavelength lamda

b = Length of radiation passed through the atomic vapor

The intensities of absorbing radiation are related to the concentration of absorbing atoms through the Beer Lambert law.

Beer, s law

$$A = \varepsilon b c$$

A is the absorbance.

2.5.3.2 Principle of Atomic Emission Spectroscopy

Atomic emission spectroscopy (AES) is different from AAS, because it utilizes the emission of radiation by a sample, rather than the absorption. For this reason samples usually have to be heated to a higher temperature by a flame or more recently a plasma. So that a greater proportion of the atoms move to an excited state. But a great care must be taken to ensure that ionization does not occur. Because the spectra from ionized atoms is different from that of non-ionized atoms. (McClements, 2003)

The relationship is that the intensity of emitted radiation at any selected wavelength is proportional to the concentration of atoms in the atomic vapor producing that particular radiation and in turn to the concentration of relevant analyte in the solution. (Pathiratne, 2004)

$$C \quad \alpha \quad I$$

Where, C= concentration of the atoms emitting radiation

I = Intensity of spontaneous emission at the selected wavelength

However analyses are carried out on relative basis due to the absence of exact relationship between concentration of analyte and the emission intensities are known to date. For this purpose, emission intensities for analytes with different standards should be measured undetr identical conditions. Because environmental factors, temperature, analyst' conditions and flame height highly affect for the accuracy of the experiment. Therefore the emission intensity of an unknown is measured under the identical conditions and then it is compared with those of the standards to measure concentration of unknown. (Pathiratne, 2004)

2.5.4 Atomic Absorption Spectrophotometer (AAS)

There are five essential components of an Atomic Absorption Spectrometer.

1. The radiation source that emits the spectrum of the element of interest. (Usually a hollow cathode lamp (HCL) and occasionally a electrode less discharge lamp (EDL)
2. An atomization device which produces the free atoms of the analyte. (a flame, furnace or a vapor generation device)
3. A monochromator for wavelength selection purpose. It allows the isolation of the resonance line from other lines produced in the spectrum of the lamp.
4. A detector, which measures the light intensity of the signal passing through the monochromator.
5. An amplifier and read out system which processes the signal from detector and converts it into a display.

2.5.4.1 The radiation sources for AAS

In most cases the radiation source is a hollow cathode lamp because it has an excellent, bright, stable line source for most elements. However an EDL is also used for some volatile elements,where low intensity and short tamp life time are a problem. EDLs are typically more intense than hollow cathode lamps and, therefore, may offer better precision and lower detection limits for some elements. (Perkin-Elmer corporation, 1996)

(a) Hollow Cathode Lamp (HCL)

It consists of a tungsten anode and a cylindrical cathode sealed in a glass cylinder filled with neon or argon. And the internal pressure is adjusted to 1 – 5 torr with the filled gas. The optimum fill gas is selected that gives the best lamp intensity while taking into consideration spectral interferences from either neon or argon. A red glow is observed in lamps filled with neon, while argon filled lamps have a blue glow.(Perkin-Elmer corporation,1996).The cathode is either fabricated from the analyte metal or else serves as a support for coating of

that metal. The glass cylinder has a quartz or UV glass window for optimum transmittance of the emitted radiation. These HCL lamps are available for more than 60 elements.

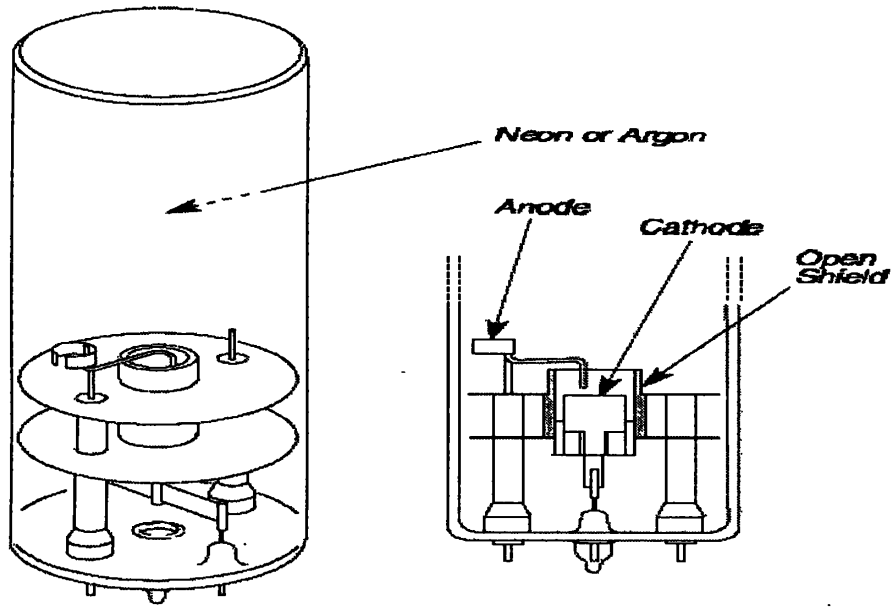

Figure: 2.5 Diagram of a hollow cathode lamp
(Source: Perkin-Elmer corporation, 1996)

The Hollow cathode emission process includes three main steps as Sputtering, Excitation and Emission. When an electrical potential of 300V is supplied between anode and cathode some of the fill gas atoms are ionized. The positively charged ions collide with the negatively charged cathode and dislodge metal atoms by producing an atomic cloud. .This process is called as "sputtering." Sputtered metal atoms are further excited and emit radiation at their characteristic wavelength through the impact with the fill gas.

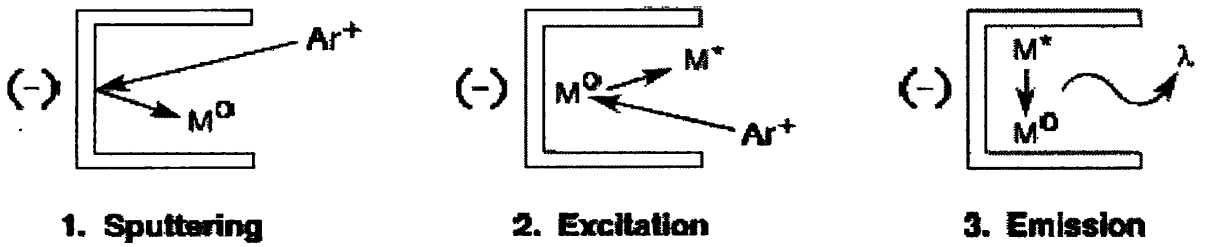

Figure: 2.6 Hollow Cathode emission process
(Source: Perkin-Elmer corporation, 1996)

Atoms producing an emission line in hollow cathode lamps are relatively at a lower temperature than those in a flame As a result emission lines in a HC lamps are broadened less than that the absorption lines in a flame.

Hollow cathode lamps have a finite lifetime. With extended use, the sputtering process removes some of the metal atoms from the cathode and these are deposited elsewhere. Fill gas is absorbed in the sputtered metal, on the glass walls and also absorbed into the glass from bombardment. Lamps for volatile elements age faster due to more rapid sputtering of the cathode. (Perkin-Elmer corporation, 1996).Some HCL are filled with cathode containing more than one element; and such lamps are known as multi-element hollow cathode lamps and they provide spectra for determination of more than one element.

(b) Electrode less Discharge Lamp (EDL)

For most elements, the hollow cathode lamp is a completely satisfactory source for atomic absorption. In a few cases, however, the quality of the analysis is impaired by limitations of the hollow cathode lamp. The primary cases involve the more volatile elements, where low intensity and short lamp life are a problem. The atomic absorption determination of these elements can often be dramatically improved with the use of brighter, more stable sources such as the "electrode less discharge lamp."(Perkin-Elmer corporation, 1996)

Electrode less discharge lamps are typically much more intense and, in some cases, more sensitive than hollow cathode lamps. Therefore they offer an analytical advantage of better precision and lower detection limits where an analysis is intensity-limited. In addition to providing superior performance, the useful lifetime of an EDL is typically much greater than that of a hollow cathode lamp for the same element. Electrode less discharge lamps are available for a wide variety of elements, including most of the volatile metals. (Perkin-Elmer corporation, 1996)

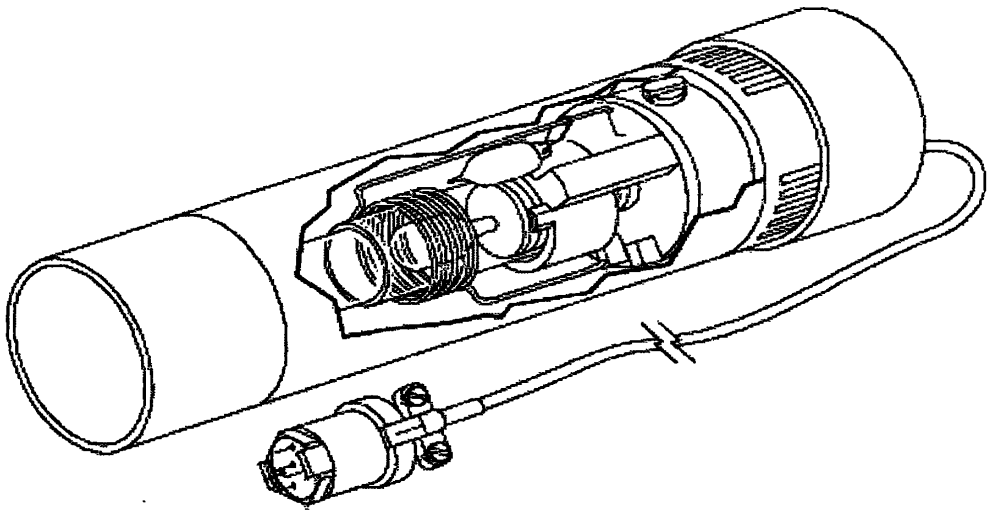

Figure: 2.7 An Electrodeless Discharge Lamp
(Source: Perkin-Elmer corporation, 1996)

2.5.4.2 The Atomization devices for AAS

A flame, furnace or a vapor generation system can be used.

(a) Flames

The flame can be produced by burning a suitable fuel in an oxidizing environment. The flame design is common for atomic emission spectroscopy even. All desolvation, atomization, and excitation occur in the flame. Other flame designs nebulize the sample and premix it with the fuel and oxidant before it reaches the burner. Atomic-absorption instruments almost always use a nebulizer and also use a slot burner to increase the path length for the sample absorption. (Tissue, 1996)

Table 2.4 some fuel-oxidant combinations used to produce analytical flame with their approximate temperatures.

Fuel	Oxidant	Ignition Temperature °C	Approximate maximum Temperature °C
H_2	Air	530	2045
H_2	O_2	450	2660
C_2H_2	Air	350	2125
C_2H_2	O_2	335	3100
C_2H_2	N_2O	400	2935

(Source: Pathiratne, 2004)

Nebulizer Burner system.

The gas flow which is under pressure passes into nebulizer. The gas is then expanded through venturi throat and develops a pressure difference which draws a flow of sample solution through the inlet capillary. This solution emerging at a very high speed is then shattered into droplets ranging in size from 1-2 to 100 or more micrometers.

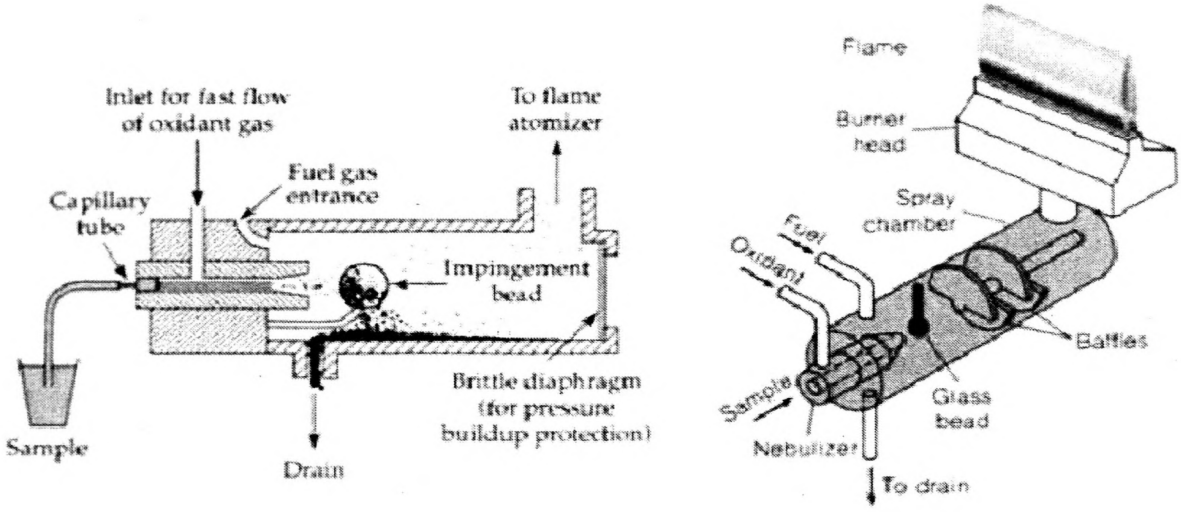

Figure: 2.8 A diagram of a Nebulizer and the process of nebulization
(Source: New Mexico state university, 2006)

Then this spray of droplets passes into spray chamber. In the spray chamber droplets and the oxidant gas mix with the fuel gas. In this case a set of baffles makes precipitation of the more larger and heavier droplets of solution and these are then drained from the chamber to waste.

The Atomization process in flame

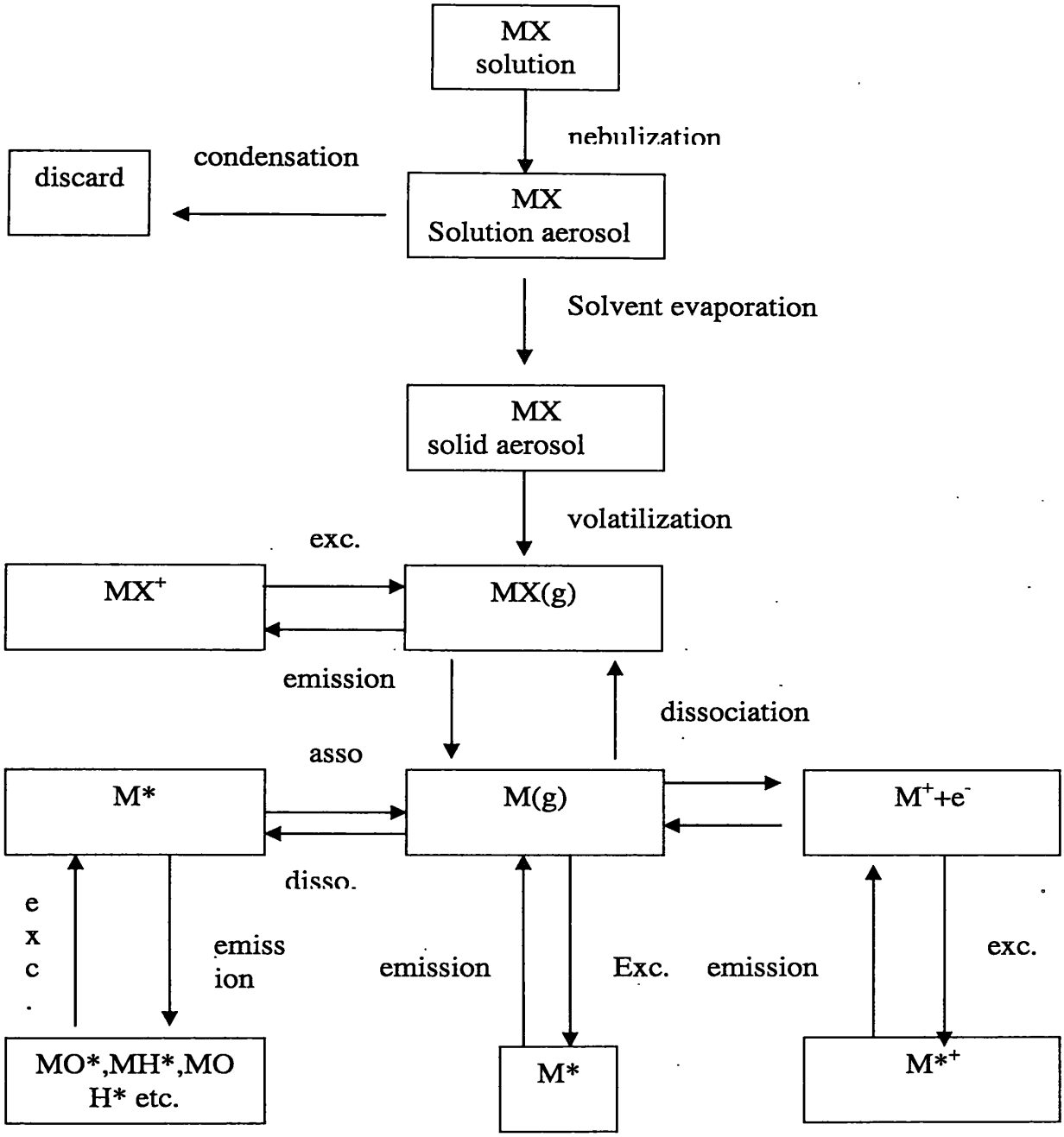

Figure: 2.9 Atomization process occurring in a flame

(Source: Pathiratne, 2004)

Exc. = excitation

M* = excited M

Ionization, association and excitation processes lead to reduction in concentration of free atoms M(g) .This M(g) is the required species for atomic absorption and emission spectrometry.

2.5.4.3 Interferences in Atomic Absorption Spectroscopy

Two types of interferences are encounted in atomic absorption spectroscopy.

(a) Spectral Interferences

Includes any physical process that affects the light intensity at the analytical Wavelength.

(i) Absorption of source radiation

An element in the sample other than the element of interest may absorb at the wavelength of the spectral band being used. Such interference is very rare because emission lines from hollow cathode lamps are so narrow. So that only the element of interest is capable of absorbing the radiation; e.g.: interference of iron in zinc determination. This problem is avoided by narrowing slit width or by selecting another resonance line. Removing the contaminant from the absorption cell is also applicable.

(ii) Background Absorption of source radiation

Occurs due to scattering the source radiation by the particulates present due to incomplete atomization, thereby attenuating the radiation reaching the detector. This problem can be overcome by using a higher flame temperature to ensure complete atomization of the sample Also can be used alternative background correction devices.

(b) Non Spectral Interferences

Includes flame interferences which ours due to incomplete flame atomization process

(i) Transport Interferences

Occurs due to the all factors in the sample solution affecting the rate of aspiration, nebulization or transport into the flame. Such factors are viscosity, surface tension, density and the vapor pressure of the sample solution. Transport interference can be overcome by matching as closely as possible the physical properties of the sample solution and the using standards.

(ii) Evaporation interferences

Arise from alterations in the rate of evaporation of solid aerosol in the flame. This may be either specific or non-specific to particular elements. Specific interference occurs when an

interferent combines with the element of interest to form a compound of low volatility. This yields a falsely low result because some of the element remains unatomized in the flame. (E.g. effect of PO_4^- anion in Calcium.) Non-specific interference occurs when the analyte occurs in a solution of high total solids. One approach for overcoming this interference is to add another element to the sample and reference that will compete with the analyte for compound formation. Other strategies are to use a higher temperature flame and to add ligand such as EDTA which will complex the analyte and prevent it from reacting with the interferant.

(iii)Ionization Interference

Arise from shift in the dissociation and association equlibria.

$$M \rightleftharpoons M^+ + e^-$$

Ionization increases with increasing flame; hence it is not a problem in air-acetylene gas flow.But it can be a problem in N_2O-acetylene flame. To reduce this ionization interference reagents are added which are called ionization suppressers. They are easily ionized before the element of interest and increase the electrons concentration in the media. So then the backward reaction occurs following the Le-Chatalier principle.

2.5.4.4 Elimination of Interferences and Optimization of Flame atomization

The detection and elimination above interferences largely depends on the ingenuity and experience of the analyst. However there are some approaches that may be useful.

1. Prepare standards that imitate the sample composition. But if the composition varies from sample to sample then the standard addition method should be used.
2. By addition of a relatively large concentration of the interferent species to both samples and standards. So that the variation is "buffered" It is possible to add a large amount of easily ionizable species to suppress ionization. (e.g.: Lanthanum for Calcium)
3. Choose of a flame with higher temperatures will reduce the compound formation in the flame and reduce the effect of slow evaporation steps.
4. Flame gas composition and gas phase equlibria also affects the efficiency of atomization. Many elements form stable mono-oxides in flame and substantial gain in atomic concentration can be obtained by increasing the fuel amount in the flame mixture, there by reducing the free O_2 concentration in the flame resulting free atoms.

$$MO \longrightarrow M + O$$

2.5.4.5 Types of AAS

Two main types of AAS can be identified.

(a) Single Beam Spectrophotometer

With single-beam systems, a short warm up period is required to allow the source lamp to stabilize.

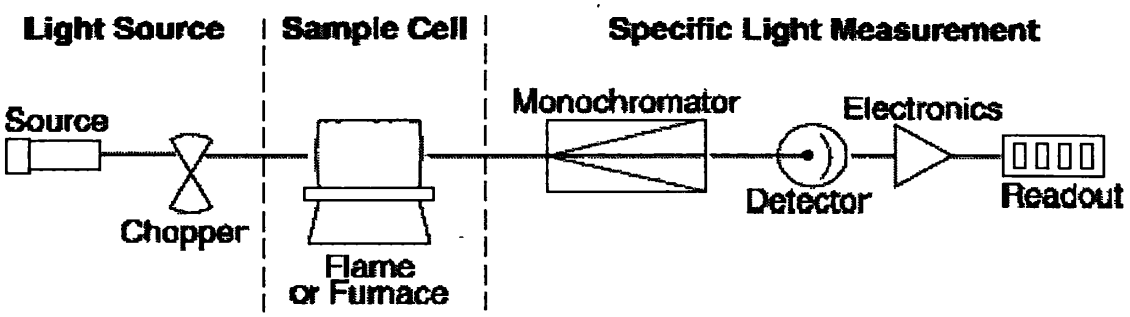

Figure: 2.10 Schematic block diagram of single-beam spectrophotometer
(Source: Perkin-Elmer corporation, 1996)

(b) Double Beam Spectrophotometer

The light from the source lamp is divided into a sample beam, which is focused through the sample cell, and a reference beam, which is directed around the sample cell. In a double-beam system, the readout represents the ratio of the sample and reference beams. Therefore, fluctuations in source intensity do not become fluctuations in instrument readout, and stability is enhanced. Generally, analyses can be performed immediately with no lamp warm-up required. (Perkin-Elmer corporation, 1996)

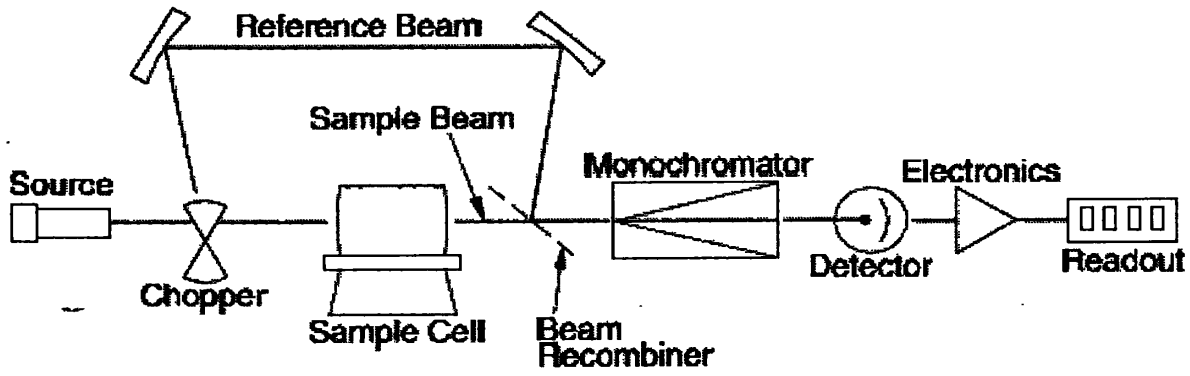

Figure 2.11 Schematic block diagram of Double-Beam Spectrophotometer
(Source: Perkin-Elmer corporation, 1996)

CHAPTER 03

03 Materials and Methodology

3.1 Materials

3.1.1 Instruments

Flame Atomic Absorption Spectrophotometer: VARIAN SpectrAA

Muffle furnace: CARBOLIRA (GLM)

Analytical balance: METTLER AJ 100

Water bath

3.1.2 Equipments

Funnel: Borosilicate glass

Glass beaker (100 ml)

Measuring cylinder (20 ml)

Volumetric flasks (50ml,100ml,250ml,500ml and 1L) :MBC BS1792 ISO1042 ENGLAND

Crucibles : HALDENWANGER

Thermometer : WITEC Germany

Qualitative filter papers: Whatmann

Pipette

Pipette filler

Spatula

Watch glass

Sampling equipments (agitators,triers or plungers)

Glass rod

Micropipette

3.1.3 Reagents

3.1.3.1 Standard solutions

Sodium (Na) standard solution (1000 ppm)

Potassium (K) standard solution (1000 ppm)

Iron (Fe) standard solution (1000 ppm)

Calcium (Ca) standard solution (1000 ppm)

3.1.3.2 Chemicals

0.1M HNO3

Lanthanum nitrate

Diammonium hydrogenphospate [$(NH_4)_2HPO_4$]

Sodium chloride (NaCl)

Potassium chloride (KCl)

1% KCl solution

Conc. HCl

Trichloro acetic acid (TCA)

Calcium Carbonate ($CaCO_3$)

Distilled water

1% $CsNO_3$ solution

3.2 Methodology

3.2.1 Sampling

All samples were collected randomly from the market according to standard sampling procedures.

3.2.1.1 Raw milk and other liquid milk products

Milk was thoroughly mixed by inverting, stirring or plunging until sufficient homogeneity is obtained using a stirrer and sampling was done using a dipper. (ISO, 707)

3.2.1.2 Dried milk powders

Sampling was avoided on rainy days or when humidity is high, so as to reduce moisture absorption from air. Homogeneity was obtained by shaking or alternatively rolling and inverting the container before opening it. A clean, dried "borer" was used for sampling milk powders. (AOAC, 2002)

3.2.1.3 Butter

A clean, dried butter trier of sufficient length to pass diagonally to the bottom of the container was used for sampling. Sample size should not be less than 50g and it is recommended to use opaque sample containers and wrap the container in Al foil. (ISO, 707)

3.2.1.4 Cheese

A knife with a smooth surface was used for sampling among the different sampling techniques. And a spatula was used in weighing the cheese sample.

3.2.1.4 Fermented dairy products (Yoghurt, Curd)

Before taking the reference sample the sample container was opened and thoroughly mixed using a plunger. And the reference sample was taken after making the composite sample. (ISO, 707)

3.2.1.6 Butter Oil (GHEE)

An agitator and a spatula was used for sampling and the capacity of the sample container shall be such that they are almost completely filled by the sample and allow proper mixing of the content before testing. (ISO, 707)

3.2.2 Analysis of Na, K, Ca and Fe in milk powders

3.2.2.1 Preparation of ash

Different milk powder samples, which come under different trade names available in the market were selected (e.g.: full cream, non-fat, malted and infant formula) for the analysis. Laboratory sample was taken after making the container homogeneous. Then 5g of the sample were measured into clean and oven dried crucible. Then the crucible was placed in the muffle furnace for the ashing. Temperature of the furnace was adjusted to 450^0C.The initial temperature of the furnace was around 100^0C.And the temperature was increased up to 450^0C in a rate of 50^0C/hr.Ashing was carried out about 6-8 hours until the sample is completely ashed; i.e., ash should be white/grey of slightly colored. After complete ashing filtration procedure was applied.

3.2.2.2 Preparation of Ash solution

The ashed sample was dissolved using 0.1M HNO_3 and filtered into the volumetric flask (may be 50 or 100 ml) through Whatman qualitative filter paper and the funnel. After filtration was fully completed, distilled water was added to the volume. For Ca, ash was filtered into 250ml volumetric flask and 10ml $La(NO_3)_3$ was added during test portion preparation.

(*Note: This method is a deviation of AOAC 990.23 in 2002.According to AOAC method milk sample(5g) was dissolved in 20ml 40-50^0C warm water, cooled at 20^0C for few minutes and diluted to volume. This solution was then directly aspirted to Flame AAS. But the problem found was the accumulation of organic particles and this blockage of particles disturbs the performance of the instrument. Therefore an in house method was applied for the analysis.)

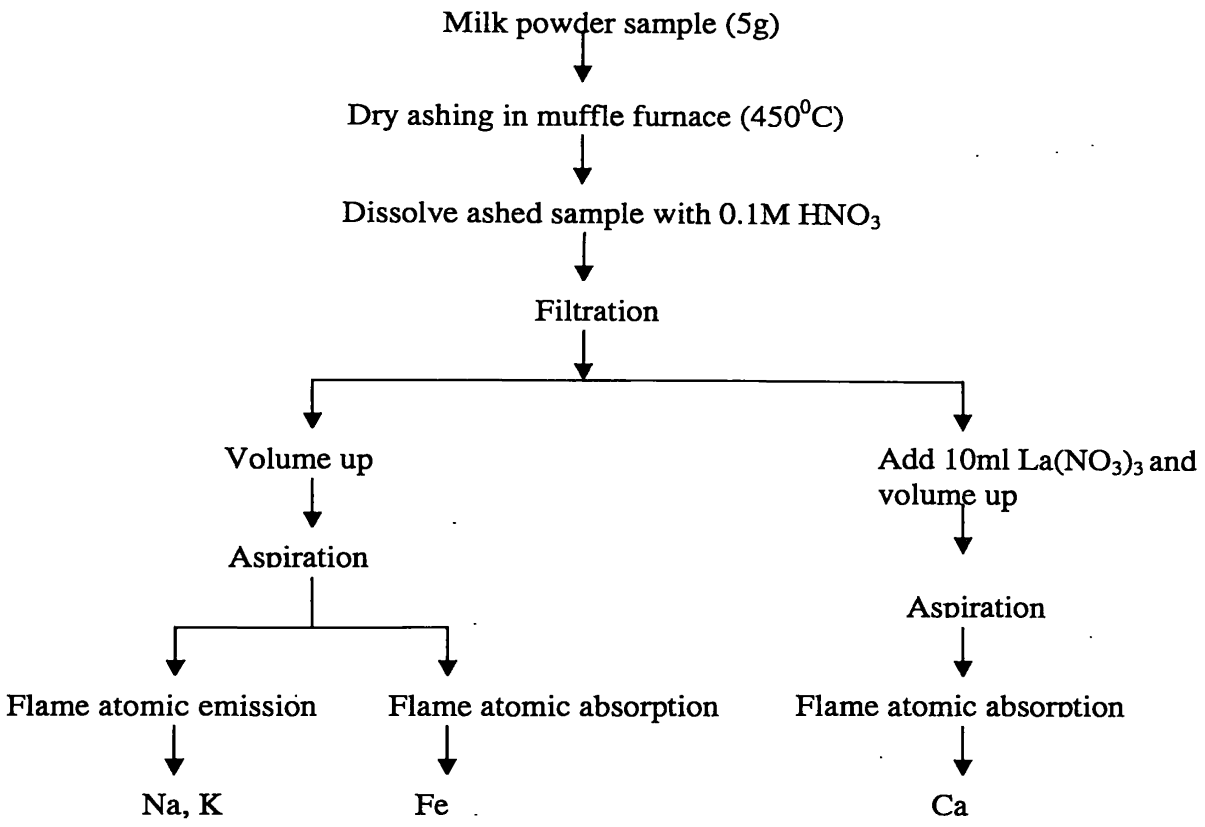

Figure: 3.1 process flow chart for analysis of Na, K, Ca and Fe in milk powders

3.2.2.3 Preparation of standard solutions

- For analysis of Na and K a bracketing system was applied. Hence a series of standards were prepared. For that standard solutions of Na, K and P were prepared as follows(AOAC 990.23,2002)

(1) Sodium(Na) standard solution

0.2542g of dried NaCl was dissolved in distil water in a 250ml volumetric flask and it was diluted to volume. The resulted standard solution was 400ppm.

(2) Potassium (K) standard solution

0.4767g of dried KCl was dissolved in distil water in a 250ml volumetric flask and it was diluted to volume. The resulted standard solution was 1000ppm.

(3) Phosphorous (P) standard solution

2.665g of diammonium hydrogenphosphate [$(NH_4)_2HPO_4$] was dissolved in distil water in a 250ml volumetric flask and it was diluted to volume. The resulted standard solution was 2500ppm

Using these standard solution series of reference solutions were prepared into six 100ml volumetric flasks as follows.

Table 3.1 Preparation and composition of Na and K reference solutions

Reference solution	Na standard solution volume taken(ml)	Na reference solution concentration (ppm)	K std.solution volume taken,(ml)	K ref.solution concentration (ppm)
1	1.5	6	5.0	50
2	2.0	8	4.5	45
3	2.5	10	4.0	40
4	3.0	12	4.0	40
5	4.0	16	3.5	35
6	5.0	20	3.0	30

For each reference solution 1ml of P standard solution was added.

(Source: AOAC 990.23, 2002)

For analysis of Ca, reference solutions of 1, 2 and 3 ppm were prepared using 1000ppm standard solution. 10ml of La $(NO3)_3$ was added to each reference solutions as the matrix modifier. And for analysis of Fe reference solutions of 1, 2 and 3 ppm were prepared using 1000ppm standard solution.

3.2.2.4 Analysis

Flame atomic absorption spectrophotometer was optimized before analysis of four metals. Then after Na and K were analyzed using Flame atomic emission and Ca and Fe were analyzed using Flame atomic absorption in the testing samples. Sample dilutions were carried according to the requirements.

3.2.3. Analysis of Na, K, Ca and Fe in other dairy products(Butter, Butter Oil, Margarine, Curd, Yoghurt, Ice cream and Liquid Milk products with out Cheese)

3.2.3.1 Preparation of samples

Samples of 5g from other dairy products (with out cheese) were measured into crucibles and sample digestion step was carried out before dry ashing.Samples were placed on the water bath for complete sample digestion and after digestion they were placed in muffle furnace for ashing purpose at not more than 450^0C .Then after the ashed samples were dissolved using 0.1M HNO_3 and filtered into volumetric flasks through Whatman qualitative filter paper and the funnel. Then distil water was added to the volume.

Sample preparation procedure of liquid milk products was different from other milk products. Hence they are liquids they did not require an ashing procedure. So 5ml of the liquid milk sample was measured into a 50ml volumetric flask and it was diluted to the volume. Then Trichloroacetic acid (TCA) was added to precipitate the milk proteins. Soon after adding TCA could observe the coagulation of milk proteins. Then the supernatant was filtered into a 50ml flask and used for direct aspiration for metal analysis. For analysis of Ca same procedure mentioned in 3.2.3.1 was applied in test portion preparation.

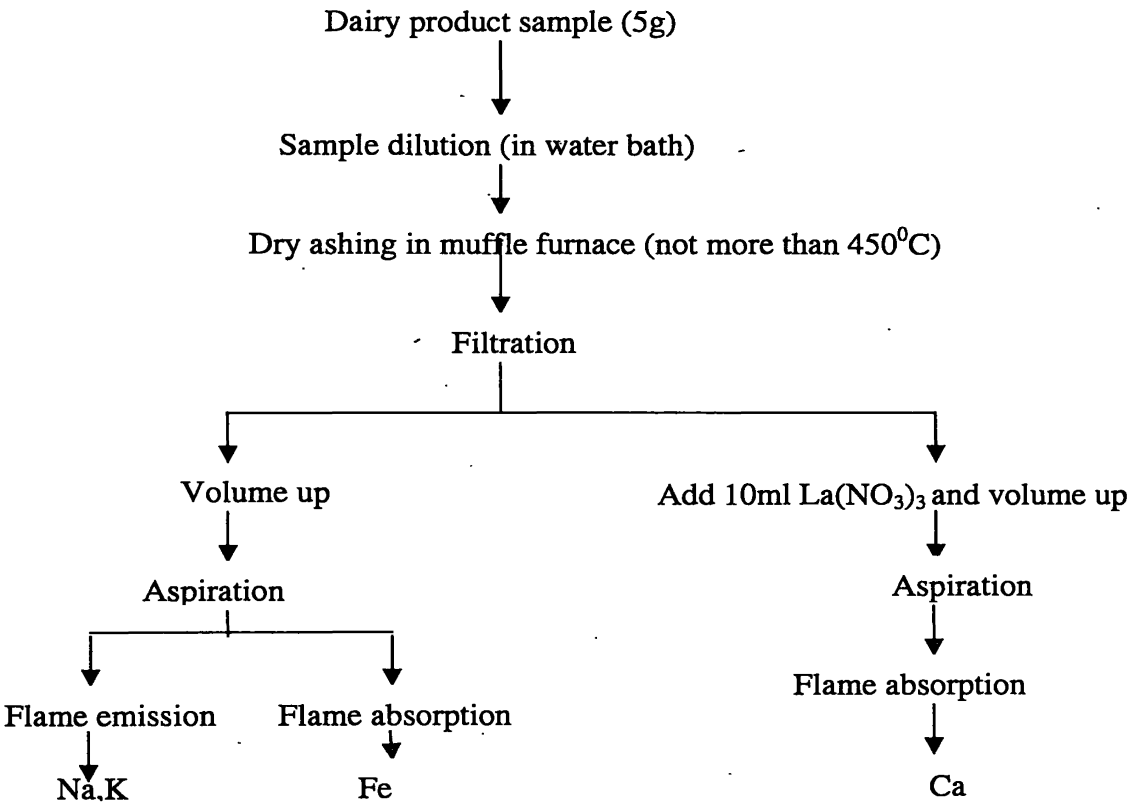

Figure: 3.2 Process flow chart for analysis of Na, K, Ca and Fe in other dairy products (Butter, Butter Oil,Curd, Yoghurt, Ice cream & liquid milk products with out Cheese)

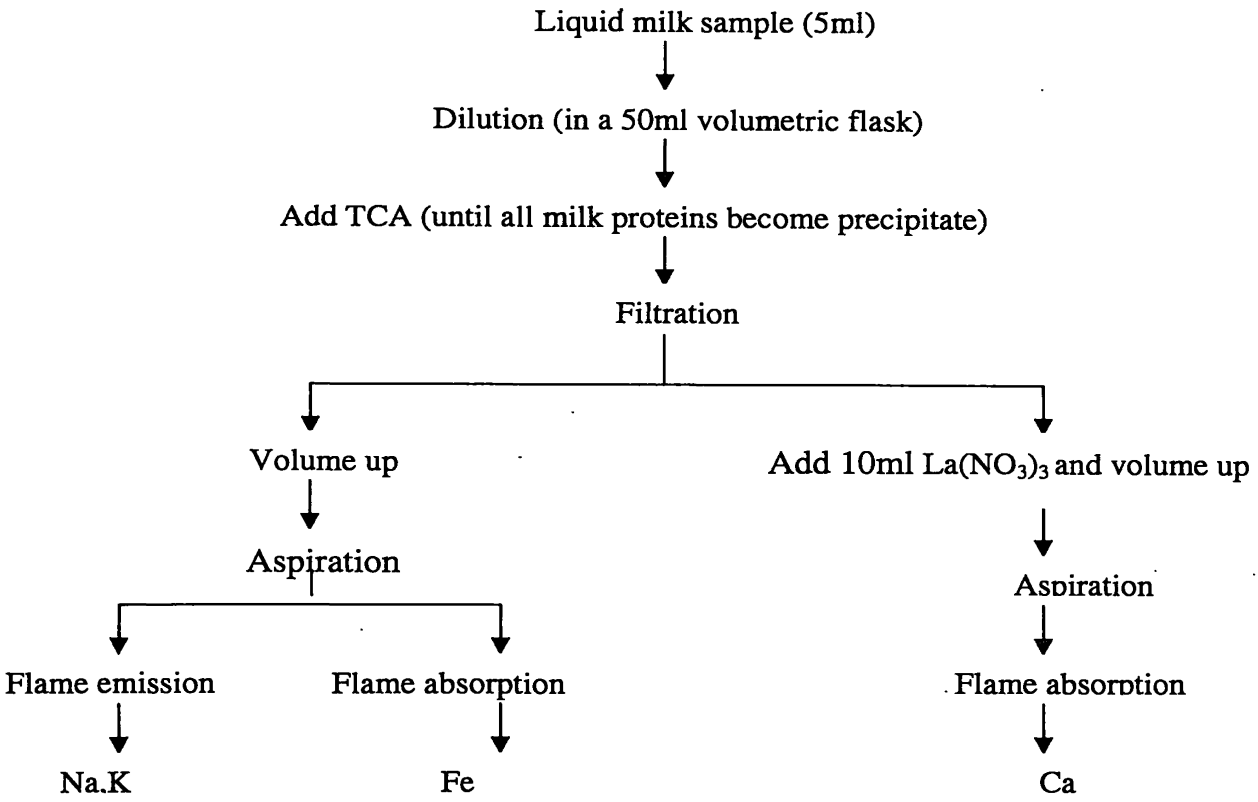

Figure: 3.3 Process flow chart for sample preparation of liquid milk products for analysis of Na, K, Ca and Fe

3.2.3.2 Preparation of standards

- For analysis of Na and K

For both Na and K standard solutions of 1, 2, 3 ppm were prepared using original standard solutions of 1000ppm.25ml of CsNO3 was added to K standards and 25ml of KCl was added to Na standards.

- For analysis of Ca and Fe

Ca standards of 1,2,3 ppm were prepared using original Ca standard solution of 1000 ppm and $La(NO3)_3$ was added as the matrix modifier for Ca standard solutions.Fe standard solutions of 1,2,3 ppm were prepared using the original Fe standard solution of 1000 ppm with out addition of matrix modifier.

3.2.3.3 Analysis

After optimization the Flame atomic absorption spectrophotometer, Na and K in the samples were analyzed using flame emission mode. Ca and Fe was analyzed using flame

absorption mode.Ca and Fe Hollow cathode lamps were used for analysis of Ca and Fe, but flame emission does not requires such lamps. Readings were taken after direct aspirating the samples.

3.2.4 Analysis of Na, K, Ca and Fe in cheese

3.2.4.1 Preparation of samples

1g of cheese sample was measured after mixing thoroughly into a crucible and placed in a water bath for digestion. After digestion was completed the crucible was kept in muffle furnace for the ashing for 16 hours at 525^{0}C and was cooled in a dessicator. Then the ash was dissolved using 1ml of 0.1M HNO_3 and filtered into 250ml volumetric flask and diluted to volume (Solution A) for analysis of Ca. Then the test portion was prepared by measuring 10ml from solution A into a 100ml volumetric flask and 10ml of $La(NO_3)_3$ was added to it and diluted to volume.(AOAC 991.25,2002).Other elements were analyzed as the same procedures mentioned above.

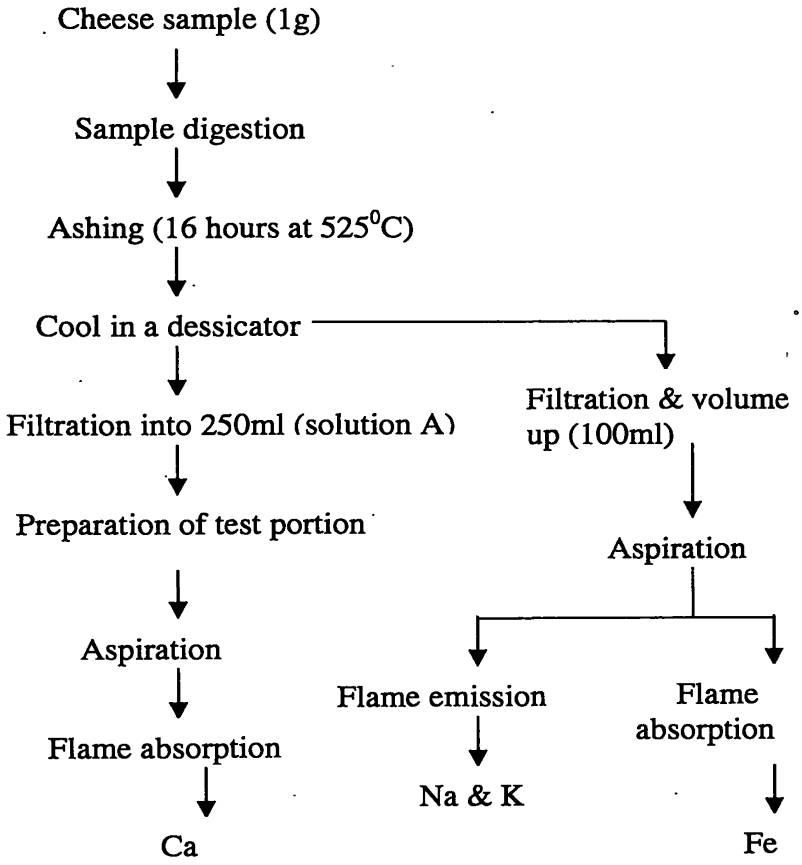

Figure: 3.4 Process flow chart for analysis of Na, K, Ca and Fe in Cheese

3.2.4.2 Preparation of standards

(A) Stock solution of $CaCO_3$

1.249g of $CaCO_3$ (dried overnight at 200^0C) was added to a 1L volumetric flask and total volume was made up to 1L with distilled water and 30ml HCl

(B) Dilute stock solution of $CaCO_3$

20ml of stock solution of $CaCO_3$ was measured to a 500ml volumetric flask and total volume was made up to 500ml with distilled water

(C) Lanthanum stock solution

5.4359g of $La(NO_3)_3$ was dissolved in 25ml 0.1M HNO_3 and diluted to volume in a 100ml volumetric flask.

(D) Working Solutions

0,5,10,15,20,25 and 30ml from the working solution of $CaCO_3$ solution was measured into a series of 100ml volumetric flasks. To each flask 10ml of Lanthanum stock solution was added and diluted to volume to obtain 0 – 6 ppm standards. Appropriate standards were used during analysis to get a linear calibration curve.

3.2.5 Test for check the accuracy of sample preparation

A mixing test was performed to check the accuracy of sample preparation. For that 5 samples of cheese were measured into 5 crucibles. Then ashing and analysis procedures were followed up as mentioned in 3.2.5. Then 5 samples were aspirated and readings were taken for Fe.

3.2.6 Recovery Tests

Recovery tests were done to examine the reliability of the test methods, which were used to analyze the above mentioned minerals in selected dairy products for the analysis. A known volume of the standard solutions were added into the samples and samples were processed according to the procedures mentioned in 3.2.3.1 and 3.2.4.1.By the way percentage recoveries were obtained for Na, K and Fe for all tested milk and milk product samples.

3.2.7 Calculations

3.2.7.1 Calculations for solid and semi-solid milk product samples(without Ca in cheese) for analysis of selected mineral

AAS reading for tested mineral = A $mgdm^{-3}$

Total volume in which sample is dispersed = B ml

Weight of sample used for ashing = Cg

%of selected mineral In the sample = $\frac{\text{(A-blank) } mgdm^{-3} \text{ x B ml x 100\%}}{\text{1000 x 1000 x Cg}}$

(*this answer should be multiply by dilution factor if there is any dilution)

3.2.7.2 Calculations for liquid milk products for analysis of selected minerals

AAS reading for tested mineral = A $mgdm^{-3}$

Total volume in which sample is dispersed = B ml

Volume of the original sample = C ml

%of selected mineral In the sample = $\frac{\text{(A-blank) } mgdm^{-3} \text{ x B ml x 100\%}}{\text{1000 x 1000 x Cml}}$

(*this answer should be multiply by dilution factor if there is any dilution)

3.2.7.3 Calculations of Ca in Cheese samples

Ca mg/100g = $\frac{\text{Z x 2500}}{\text{W x V}}$

Where;

Z = AAS reading

W = weight of cheese sample taken

V = volume of solution A taken for Assay

3.2.7.4 Calculations for Accuracy test (for Fe)

AAS reading for tested mineral = A $mgdm^{-3}$

Total volume in which sample is dispersed = B ml

Weight of sample used for ashing = Cg

%of selected mineral In the sample = $\frac{\text{(A-blank) } mgdm^{-3} \text{ x B ml x 100\%}}{\text{1000 x 1000 x Cg}}$

3.2.7.5 Calculations for Recovery tests

The selected mineral concentration in the sample = P $mgdm^{-3}$

The mineral concentration of (sample + added std.solution) = Q $mgdm^{-3}$

The added concentration of std.solution = R $mgdm^{-3}$

Recovered std.concentraion = (Q – P) $mgdm^{-3}$

Percentage recovery of the mineral = $\frac{(Q - P)\ mgdm^{-3} \times 100}{R\ mgdm^{-3}}$

CHAPTER 04

04 Results and Discussion.

4.1 Results of the analysis of Na, K, Ca and Fe in milk powders

Out of the two methods applied for analysis of Na, K, Ca and Fe in milk powder in house method was successful than AOAC method . Because from AOAC method Na, K was unable to determine due to blockage of carbon particles in the instrument, because it does not include an ashing step to separate carbon matter.

(a) Full cream

Table 4.1 Results for analysis of full cream milk powder

Brand	Na		K		Ca		Fe	
	R.V(%)	M.V(%)	R.V(%)	M.V(%)	R.V(%)	M.V(%)	R.V(%)	M.V(%)
A	0.145	0.24	0.205	1.13	0.775	0.800	0.00007	N.M
B	0.176	0.30	0.706	1.10	1.188	0.845	0.00004	N.M
C	0.130	0.29	0.702	1.31	2.0345	0.940	0.00012	N.M

R.V = Resulted value as a percentage

M.V = Mentioned value in the label as a percentage

N.M = Not mentioned in the label

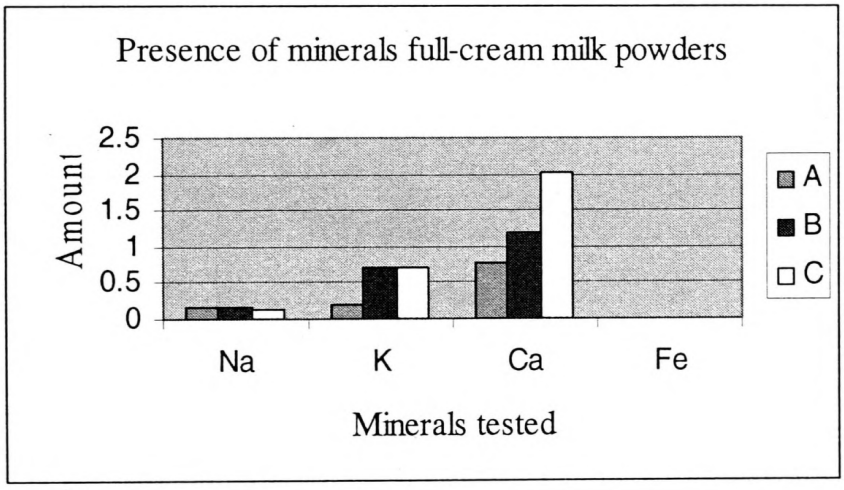

Figure: 4.1 Presence of minerals in full cream milk powders

Resulted Na content in 3 brands was approximately same. And those values are some what close but lower to the mentioned values in the labels. The difference between resulted values and mentioned values may be the errors occurred during sampling to analysis. And the K levels were very close in both B and C brands, while brand A showed a significantly different level of K other than remaining two brands. However the resulted K levels for K

were too low from that of mentioned values. But the Ca levels for two brands showed very different results from the actual value. Only the brand A showed a very close value of Ca to that of mentioned value .The only difference is -0.025%from the mentioned value and this loss may be due to the defects happened from sampling to aspiration including instrumental errors. On the other hand Fe content in three brands was in very minute quantities. Hence they were less than 0.06% can be considered as not detected. That may be the reason that the manufacturers have not mentioned the Fe content in the nutritional labels in their products.

Correlations could be seen in between R.V% and M.V% for Na, K and Ca .And the Regression equations were created to see the relationship in between R.V% and M.V%. (Appendix I)

(b) Non-fat

Table 4.2 Results for analysis of Non fat milk powder

Brand	**Na**		**K**		**Ca**		**Fe**	
	R.V(%)	M.V(%)	R.V(%)	M.V(%)	R.V(%)	M.V(%)	R.V(%)	M.V(%)
A	0.345	0.37	1.32	1.760	1.210	1.340	0.00024	N.M
B	0.329	0.35	1.29	1.745	1.279	1.427	0.00004	N.M

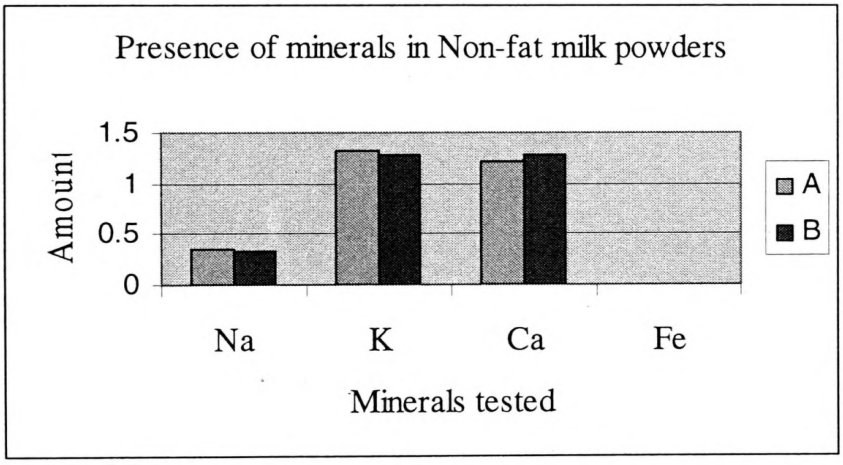

Figure: 4.2 Presence of minerals in Non-fat milk powders

Progressively the selected two brands of non fat milk powders have shown very close results for Na and Ca to that of mentioned values of the nutritional labels. Resulted values for K have deviated from around 0.4% from the mentioned value. But the resulted values for K were very close to each other and by that can prove that the procedure applied for the analysis of K have been applied accurately.

(c) Malted milk powder

Table 4.3 Results for analysis of Malted milk powder

Brand	Na		K		Ca		Fe	
	R.V(%)	M.V(%)	R.V(%)	M.V(%)	R.V(%)	M.V(%)	R.V(%)	M.V(%)
A	0.344	0.45	0.56	0.85	1.279	0.32	0.0002	N.M
B	0.0546	0.59	0.10	1.01	0.98	0.57	0.0014	N.M

Among the two brands only the A brand showed close values for Na and K.

(d) Infant formula

Table 4.4 Results for analysis of Infant formula

Brand	Na		K		Fe	
	R.V(%)	M.V(%)	R.V(%)	M.V(%)	R.V(%)	M.V(%)
A	0.2799	0.200	0.709	1.080	0.002137	N.M
B	0.2104	0.220	0.821	0.950	0.00065	N.M

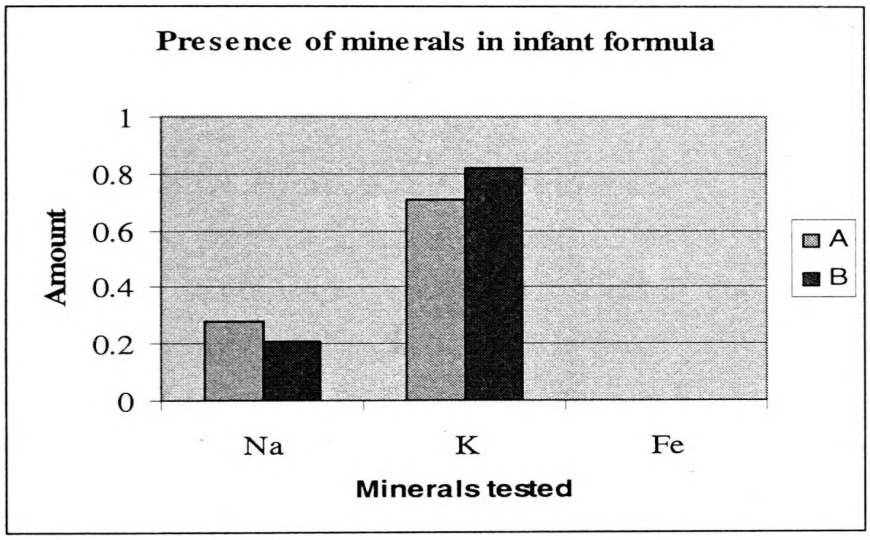

Figure 4.3 Presence of minerals in infant formula

Resulted values for K were low and very close to the mentioned value. Both brand A and B showed very close values for Na levels, but among that only brand B showed a lower value from that of mentioned value. Could not analyze Ca due to the unavailability of La $(NO_3)_3$.

(e) Full cream milk powders enriched with Calcium

Table 4.5 Results for analysis of milk powders enriched with Calcium

Brand	Na		K		Ca		Fe	
	R.V(%)	M.V(%)	R.V(%)	M.V(%)	R.V(%)	M.V(%)	R.V(%)	M.V(%)
A	0.32	0.36	0.178	1.72	1.736	2.0	0.00008	N.M

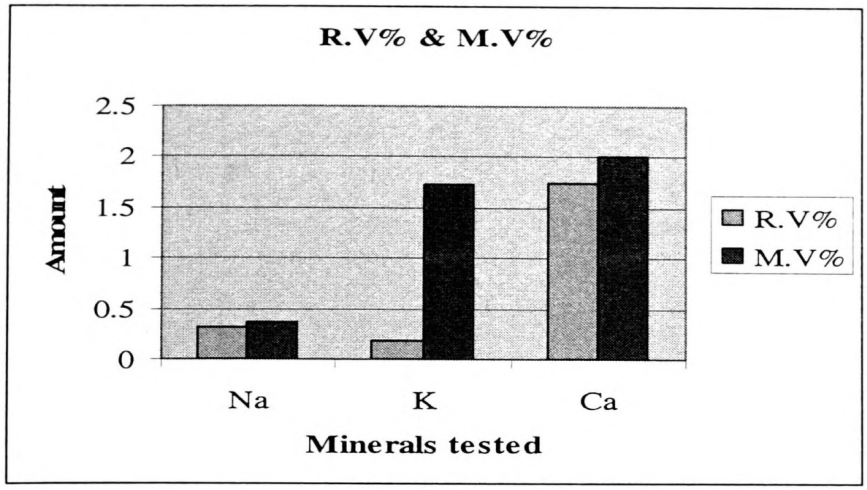

Figure: 4.4 Difference between R.V% and M.V%

Only one brand of this category was selected for the analysis. Na and Ca levels showed much closed values to that of mentioned values. This is a full cream milk powder enriched with Ca available in the market. Resulted value for Ca was 1.736% and the mentioned value was 2.0%.The difference may be due to the errors occurred from sampling to aspiration of sample.

According to the statistical approach there is a significant difference in between the median values of resulted values and mentioned values for Na and K in full cream milk powder category. But the mean values for R.V% and M.V% for Ca is same. In case of non fat milk powder significant differences could be seen only for K and Ca. But significant differences could be seen in between mean values of R.V% & M.V% for Na, K and Ca. As well as in infant formula significant difference could be seen for Na. (Appendix II)

4.2 Results of the analysis of Liquid milk products

Table 4.6 Results for analysis of Liquid milk products

Category	Resulted Na%	Resulted K%	Resulted Ca%	Resulted Fe%
Sterilized milk	0.0802	0.258	1.0254	0.00014
UHT treated milk	0.0498	0.716	1.2210	0.00072

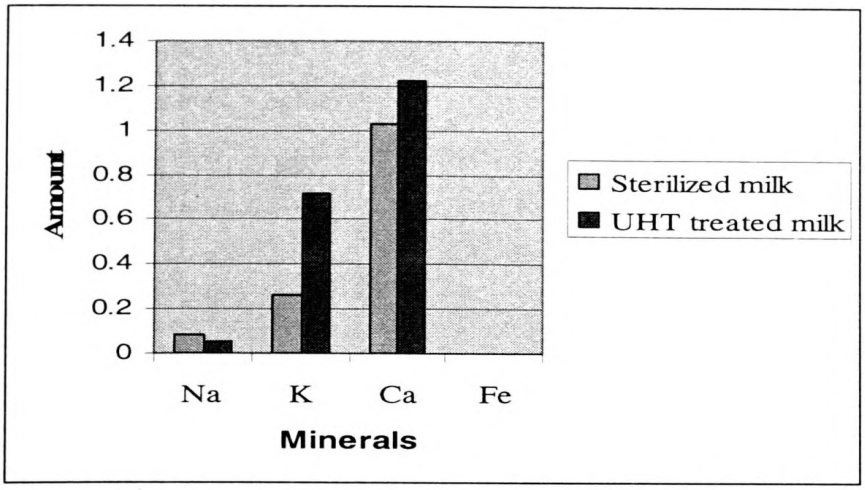

Figure: 4.5 Distribution of minerals in Sterilized milk & UHT treated milk

4.3 Results of analysis of Cheese

Table 4.7 Results for analysis of cheese

Brand	Resulted Na%	Resulted K%	Resulted Ca(mg)	Resulted Fe%
A	0.17	0.070	376.625	0.00017
B	0.109	0.046	274.108	0.00069

The results obtained for two processed cheese types were comparatively different.

4.4 Results of analysis of other dairy products

Categories

4.4.1 Curd

Table 4.8 Results for analysis of Curd

Brand	Resulted Na%	Resulted K%	Resulted Ca%	Resulted Fe%
A	0.0380	0.06	1.44	0.00034
B	0.0356	0.072	1.41	0.00027
C	0.0411	0.079	1.09	0.0003

According to the statistical approach the median Na%, K%, Ca% & and Fe% values are same for the all A, B, C brands of Curd. (Appendix III).

4.4.2 Yoghurt

4.4.2.1 Low Fat Yoghurt

Table 4.9 Results for analysis of low fat Yoghurt

Brand	Resulted Na%	Resulted K%	Resulted Ca%	Resulted Fe%
A	0.016	0.0579	0.938	0.00021
B	0.018	0.055	0.929	0.00002
C	0.0436	0.0873	0.956	0.0095

The statistical approach proved that there is no a significant difference in between median Na%, K%, Ca% and Fe% values among the selected three brands. (Appendix III)

4.4.2.2 Non-Fat Yoghurt

Table 4.10 Results for analysis of non fat Yoghurt

Brand	Resulted Na%	Resulted K%	Resulted Ca%	Resulted Fe%
A	0.0172	0.054	0.931	0.00019

The important fact is that there is no any significant difference among the two yoghurt categories for distribution of Na, K, Ca and Fe in their products. (Appendix III)

4.4.3 Ice cream

4.4.3.1 Simple Ice cream

Table4.11 Results for analysis of Simple Ice cream

Brand	Resulted Na%	Resulted K%	Resulted Ca%	Resulted Fe%
A	0.498	0.088	1.14	0.00012
B	0.0314	0.045	1.142	0.00064
C	0.0215	0.031	1.0808	0.00070

According to the statistical approach the median Na%, K%, Ca% & and Fe% values are same for the selected A, B, C brands of Simple ice cream.(Appendix III) .The selected three brands are the most popular simple ice cream brands among consumers. So the three brands resulted median Na, K, Ca and Fe values with out significant difference. (Appendix III)

4.4.3.2 Complex Ice cream

Table 4.12 Results for analysis of Complex Ice cream

Brand	Resulted Na%	Resulted K%	Resulted Fe%
A	0.0426	0.1335	0.00057
B	0.0419	0.1459	0.00048

The Statistical approach proved that there is no any significant difference among the selected two brands of complex ice cream for tested minerals. (Appendix III)

4.4.3.3 Novelties

Table 4.13 Results for analysis of Novelties

Brand	Resulted Na%	Resulted K%	Resulted Fe%
A	0.0397	0.1287	0.00040
B	0.0421	0.1405	0.00037

The Statistical approach proved that there is no any significant difference among the selected two brands of Novelties for tested minerals. And the most important fact that proved by the statistical approach is that there is no any significant difference among the three ice cream categories for the distribution of Na, K, Ca and Fe among the products. (Appendix III)

4.4.4 Butter, Butter oil and margarines

Table 4.14 Results for analysis of Butter, Butter oil and Margarines

Category	Resulted Na%	Resulted K%	Resulted Ca%	Resulted Fe%
Butter	0.812	0.0086	0.22	0.00009
Butter oil	0.002	0.0024	0.11	0.000098
Table margarine	0.1116	0.0062	0.09	0.000081
General purpose margarine	0.2827	0.0737	0.320	0.002

4.5 Results of the accuracy test

Table 4.15 Results for the accuracy test

Test no	Result obtained
Test 1	0.000143
Test 2	0.000149
Test 3	0.000125
Test 4	0.000093
Test 5	0.0001173

(See section 3.2.7 for details)

A normality test was conducted to check whether the data collected are normally distributed. (Appendix IV).According to that the sample preparation step does not affect for the final result. It means the step of sample preparation has been applied equally for each and every product throughout the analysis.

4.6 Results of the Recovery test

Table4.16 Results for the Recovery test

Sample	Recovery %		
	Na	K	Fe
Full cream milk powder	68.47	56.39	85.74
Non-fat milk powder	78.51	65.20	84.34
Malted milk powder	55.27	60.24	79.37
Infant formulae milk powder	67.27	68.50	80.34
Full cream milk powder enriched with Ca	89.30	62.25	78.05
Cheese	54.30	58.67	80.64
Non fat yoghurt	78.97	83.64	88.94
Low fat yoghurt	75.61	83.31	79.69
Simple ice cream	75.20	78.29	77.84
Complex ice cream	72.29	70.39	78.29
Novelties	70.25	70.94	71.08
Butter	58.20	64.20	69.97
Butter oil	54.28	55.20	54.09
Curd	78.09	80.21	88.21
Liquid milk products	80.27	81.09	84.89

(See section 3.2.7 for details)

According to the recovery test 7 samples for Na, 5 samples for K and 14 samples for Fe showed more than 75% of recovery percentage for the whole analysis out of 15 dairy samples. The recovery test was not able to carry out for Ca due to the unavailability of required chemicals. Full cream milk powder enriched with Ca sample showed the highest recovery% of 89.30 for Na. Low fat yoghurt category showed the highest recovery% of 83.64 for K while non-fat yoghurt category showed the highest recovery% of 88.94 for Fe. Butter oil sample has resulted very low recovery percentages for all three minerals. During the analysis of Butter oil around 50% of loss of Na, K and Fe has been occurred.

CHAPTER 05

05 Conclusion

The most suitable method for analysis milk powder is the method that deviated from AOAC method which was a combination of AOAC method and an in house method. This developed method gave results which were very close to the mentioned values in the labels for Ca in full cream milk powder, Na in Non fat milk powder and Ca in full cream milk powder enriched with Ca.

The recovery percentages obtained for milk products were as follows. For Na 55 – 95%, K 56 – 70% and 78 – 86% for Fe. So this method is acceptable for analysis of Na, K, Ca and Fe in milk powders. The second method; AOAC method was not able to continue due to the blockage of carbon particles. Because there is no an ashing step for separating carbon related organic matter. The loss percentages for the above three metals were around 30%.Specially the avoidance of contamination in trace analysis is critical because of the low level of the trace inorganics in foods and the ubiquitous presence of these elements in reagents, containers and air. Therefore it is very important to maintain an attitude of a 'useful paranoia' in evaluating and eliminating potential sources of contamination in every step of the analysis. These steps include initial collection of the bulk sample, making the sample into homogeneous and sample preparation.

The other most important point is that the samples used for the analysis should be very fresh. Because during storing samples in glass wares for two or more days there will be a significant loss of minerals. The standards also should prepare freshly.

According to the statistical approach applied can conclude that there is no any significant difference in between the Na%, K%, Ca% and Fe% values among the selected Low fat yoghurt, Simple ice cream, Complex ice cream and novelties brands available in the market. The difference among the Low fat and Non fat yoghurt is the amount of fat present. But the results conclude that the tested mineral composition does not depends on the fat portion present in the yoghurt. Complex ice cream and Novelties differ from Simple ice cream due to the presence of optional ingredients mentioned in chapter II from the basic ingredients of an ice cream. But the results conclude that these optional ingredients do not affect for the mineral composition of any ice cream.

In the product wise comparison based on the analyzed mineral composition can conclude that there is no any significant difference among Curd, Yoghurt and Ice cream categories. So by consuming equal amount of any Curd, Yoghurt and Ice cream variety available in the market can obtain very close mineral fraction.

CHAPTER 06

06 RECOMMENDATION

Further studies should be continued to completely apply the AOAC method for analysis of metals (especially Na and K) in milk powders as there is around 40% loss of Na and K during analysis using the developed method and the loss may be due to the ashing. Because according to the AOAC method the milk powder samples could be directly analyzed by dissolving in hot water with out ashing.Ashing is the only difference in between two suggested methods for analysis of Na and K in milk powder samples.

All the samples and standards should have to prepare freshly and have not to store in glass equipments at any time. Because there may be a significant loss of minerals during storing samples in glass equipments.

Comparison and a study could have been done about the changes occurring during processing of raw milk if the facilities were able to analyze raw milk. By that could have been check whether there is a loss of above minerals during processing raw milk into different dairy products.

The accuracy of FAAS highly depends on sample preparation. Therefore it is very important to maintain an attitude of a 'useful paranoia' in evaluating and eliminating potential sources of contamination in every step of the analysis paying a high attention for sample preparation.

The highly acceptable recovery percentages lay in between 80- 110%.So further studies should be carried out to increase the recovery percentages of the tests

It is better if would be able to analyze more than five brands for each dairy product category available in the local market to have a better comparison.

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APPENDIX I

Correlations in between resulted value and mentioned value for Na in full cream milk powder

H0: there is a correlation in between R.V% and M.V%
H1: no correlation in between R.V% and M.V%

```
Pearson correlation of R.V% and M.V% = 0.042
P-Value = 0.973
```

Interpretation: P value is > 0.05. So H0 is not rejected. So there is a strong (+)ve correlation in between R.V% & M.V% for Na in full cream milk powder.

Regression Analysis: M.V% versus R.V%

```
The regression equation is
M.V% = 0.268 + 0.06 R.V%

Predictor     Coef  SE Coef      T      P
Constant    0.2680   0.2075   1.29  0.419
R.V%         0.058    1.369   0.04  0.973

S = 0.0454205   R-Sq = 0.2%   R-Sq(adj) = 0.0%
```

Correlations in between R.V% & M.V% for K

H0: there is a correlation in between R.V% and M.V%
H1: no correlation in between R.V% and M.V%

```
Pearson correlation of R.V% and M.V% = 0.375
P-Value = 0.755
```

Interpretation:P value is > 0.05. So H0 is not rejected. So there is a strong (+)ve correlation in between R.V% & M.V% for K in full cream milk powder.

Regression Analysis in between R.V% & M.V%

```
The regression equation is
M.V% = 1.10 + 0.148 R.V%

Predictor     Coef  SE Coef      T      P
Constant    1.1006   0.2145   5.13  0.123
R.V%        0.1478   0.3655   0.40  0.755


S = 0.148914   R-Sq = 14.0%   R-Sq(adj) = 0.0%
```

Correlations: R.V%& M.V% for Ca

H0: there is a correlation in between R.V% and M.V%
H1: no correlation in between R.V% and M.V%

```
Pearson correlation of R.V% and M.V% = 1.000
P-Value = 0.005
```

Interpretation:P value is < .05. So H0 is rejected. So no correlation in between R.V% & M.V% for Ca in full cream milk powder.

Regression Analysis: M.V% versus R.V%

```
The regression equation is
M.V% = 0.713 + 0.111 R.V%


Predictor       Coef   SE Coef       T      P
Constant    0.713340  0.001146  622.69  0.001
R.V%        0.111314  0.000800  139.14  0.005


S = 0.000726445   R-Sq = 100.0%   R-Sq(adj) = 100.0%
```

APPENDIX II

Statistical approach for analyzed minerals in full cream milk powder

(1) Mann Whitney test for Na in full cream milk powder

H0: $\mu 1 = \mu 2$ Vs

H1: $\mu 1 \neq \mu 2$

Mann-Whitney Test and CI: R.V, M.V%

```
      N  Median
R.V%  9  0.1700
M.V%  9  0.2900

Point estimate for ETA1-ETA2 is -0.1200
95.8 Percent CI for ETA1-ETA2 is (-0.1650,-0.0700)
W = 54.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0062
```

Interpretation: P value 0.0062.P value is < 0.025.So H0 is rejected. So there is a significant difference in between the mean values of resulted values and mentioned values for Na.

(2) Mann Whitney test for K in full cream milk powder

H0: $\mu 1 = \mu 2$ Vs

H1: $\mu 1 \neq \mu 2$

Mann-Whitney Test and CI: R.V, M.V%

```
      N  Median
R.V%  9  0.6970
M.V%  9  1.1300

Point estimate for ETA1-ETA2 is -0.6000
95.8 Percent CI for ETA1-ETA2 is (-0.9771,-0.3890)
W = 45.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0004
The test is significant at 0.0004 (adjusted for ties)
```

Interpretation: P value is 0.0004 P value is < 0.025.So H0 is rejected. So there is a significant difference in between the mean values of resulted values and mentioned values for K

(3) Mann Whitney test for ca in full cream milk powder

H0: $\mu 1 = \mu 2$ Vs

H1: $\mu 1 \neq \mu 2$

Mann-Whitney Test and CI: R.V, M.V%

```
      N  Median
R.V%  6  1.1880
M.V%  6  0.8450

Point estimate for ETA1-ETA2 is 0.3180
95.5 Percent CI for ETA1-ETA2 is (-0.1449,1.2048)
W = 49.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.1282
The test is significant at 0.1262 (adjusted for ties)
```

Interpretation: P value is 0.1262 P value is > 0.025.So H0 is not rejected. So there is not a significant difference in between the mean values of resulted values and mentioned values for Ca.

Statistical approach for analyzed minerals in non fat milk powder

(1) **Mann Whitney test for Na in non fat milk powder**

H0: $\mu 1 = \mu 2$ Vs

H1: $\mu 1 \neq \mu 2$

Mann-Whitney Test and CI: R.V, M.V%

```
      N   Median
R.V%  6  0.33200
M.V%  6  0.36000
Point estimate for ETA1-ETA2 is -0.02800
95.5 Percent CI for ETA1-ETA2 is (-0.08800,0.05103)
W = 36.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.6889
The test is significant at 0.6847 (adjusted for ties)
```

Interpretation: P value is 0.6847 P value is > 0.025.So H0 is not rejected. So there is not a significant difference in between the median values of resulted values and mentioned values for Na

(2) **Mann Whitney test for K in non fat milk powder**

H0: $\mu 1 = \mu 2$ Vs

H1: $\mu 1 \neq \mu 2$

Mann-Whitney Test and CI: R.V, M.V%

```
      N  Median
R.V%  6  0.4500
M.V%  6  1.7525
Point estimate for ETA1-ETA2 is -1.3025
95.5 Percent CI for ETA1-ETA2 is (-1.4550,-1.2101)
W = 21.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0051
The test is significant at 0.0045 (adjusted for ties)
```

Interpretation: P value is 0.0045 P value is < 0.025.So H0 is rejected. So there is a significant difference in between the median values of resulted values and mentioned values for K

(3) **Mann-Whitney Test for Ca in non fat milk powder**

```
      N  Median
R.V%  6  1.1765
M.V%  6  1.3835
Point estimate for ETA1-ETA2 is -0.2070
95.5 Percent CI for ETA1-ETA2 is (-1.2428,-0.0971)
W = 21.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0051
The test is significant at 0.0045 (adjusted for ties)
```

Interpretation: P value is 0.0045 P value is < 0.025.So H0 is rejected. So there is a significant difference in between the median values of resulted values and mentioned values for Ca

Statistical approach for analyzed minerals in Malted milk powder

(1) Mann-Whitney Test for Na in malted milk powder

```
      N  Median
R.V%  6  0.1935
M.V%  6  0.5200

Point estimate for ETA1-ETA2 is -0.3265
95.5 Percent CI for ETA1-ETA2 is (-0.5201,-0.1079)
W = 21.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0051
The test is significant at 0.0045 (adjusted for ties)
```

Interpretation: P value is 0.0045 P value is < 0.025.So H0 is rejected. So there is a significant difference in between the median values of resulted values and mentioned values for Na

(2) Mann-Whitney Test for K in malted milk powder

```
      N  Median
R.V%  6  0.2500
M.V%  6  0.9300

Point estimate for ETA1-ETA2 is -0.6800
95.5 Percent CI for ETA1-ETA2 is (-0.9102,-0.1401)
W = 24.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0202
The test is significant at 0.0185 (adjusted for ties)
```

Interpretation: P value is 0.0185 & is < 0.025.So H0 is rejected. So there is a significant difference in between the median values of resulted values and mentioned values for K

(3) Mann-Whitney Test for Ca in malted milk powder

```
      N  Median
R.V%  6  1.1125
M.V%  6  0.4450

Point estimate for ETA1-ETA2 is 0.6370
95.5 Percent CI for ETA1-ETA2 is (0.4599,0.8270)
W = 57.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0051
The test is significant at 0.0045 (adjusted for ties)
```

Interpretation: P value is 0.0045& < 0.025.So H0 is rejected. So there is a significant difference in between the median values of resulted values and mentioned values for Ca

Statistical approach for analyzed minerals in Infant formula

(1)Mann Whitney test for Na in Infant formula

H0: $\mu 1 = \mu 2$ Vs

H1: $\mu 1 \neq \mu 2$

Mann-Whitney Test and CI: R.V, M.V%

```
      N  Median
R.V%  6  0.2210
M.V%  6  0.2100

Point estimate for ETA1-ETA2 is 0.0195
95.5 Percent CI for ETA1-ETA2 is (-0.0077,0.1571)
W = 48.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.1735
The test is significant at 0.1667 (adjusted for ties)
```

Interpretation: P value is 0.1167. P value is > 0.025.So H0 is not rejected.So there is not a significant difference in between the median values of resulted values and mentioned values for Na

(2)Mann Whitney test for K in Infant formula

H0: $\mu 1 = \mu 2$ Vs

H1: $\mu 1 \neq \mu 2$

Mann-Whitney Test and CI: R.V, M.V%

```
      N  Median
R.V%  6  0.7525
M.V%  6  1.0150

Point estimate for ETA1-ETA2 is -0.2500
95.5 Percent CI for ETA1-ETA2 is (-0.3700,-0.1050)
W = 24.0
```

```
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0202
The test is significant at 0.0185 (adjusted for ties)
```

Interpretation: P value is 0.0185. P value is < 0.025.So H0 is rejected.So there is a significant difference in between the median values of resulted values and mentioned values for K

APPENDIX III

Statistical approach for other dairy products

(1) Kruskal Wallis test for Curd varieties for Na

```
Kruskal-Wallis Test on Na%
H0:all the treatment medians are same          Vs
H1:at least one is different from others
                        Ave
Curd      N    Median   Rank      Z
1         3   0.31100    8.0   2.32
2         3   0.03300    2.7  -1.81
3         3   0.04080    4.3  -0.52
Overall   9              5.0
H = 5.96  DF = 2   P = 0.051
```

Interpretation:P value is 0.051 and is > 0.05.So H0 is not rejected. So the median Na% levels are same for A, B,C brands.

(2) Kruskal Wallis test for Curd varieties for K

```
Kruskal-Wallis Test on K%
H0:all the treatment medians are same          Vs
H1:at least one is different from others
                      Ave
Curd      N    Median  Rank      Z
1         3   0.06000   4.3  -0.52
2         3   0.06800   4.0  -0.77
3         3   0.08000   6.7   1.29
Overall   9             5.0
H = 1.69  DF = 2   P = 0.430
```

Interpretation:P value is 0.430 and is > 0.05.So H0 is not rejected. So the median K% levels are same for A, B, C brands.

(3) Kruskal Wallis test for Curd varieties for Ca

```
Kruskal-Wallis Test on Ca%
H0:all the treatment medians are same          Vs
H1:at least one is different from others
                      Ave
Curd      N  Median  Rank      Z
1         3  1.5300   6.7   1.29
2         3  1.3900   6.0   0.77
3         3  0.9900   2.3  -2.07
Overall   9           5.0
H = 4.36  DF = 2   P = 0.113
```

Interpretation:P value is 0.113 and is > 0.05.So H0 is not rejected. So the median Ca% levels are same for A, B,C brands.

(4) Kruskal Wallis test for Curd verities for Fe

```
Kruskal-Wallis Test on Fe
H0:all the treatment medians are same          Vs
H1:at least one is different from others

                         Ave
Curd      N      Median  Rank      Z
1         3  0.0003200   6.0   0.77
2         3  0.0003100   4.8  -0.13
3         3  0.0002000   4.2  -0.65
Overall   9              5.0

H = 0.69  DF = 2   P = 0.709
H = 0.69  DF = 2   P = 0.707  (adjusted for ties)
```

Interpretation:P value is 0.707 and is > 0.05.So H0 is not rejected. So the median Fe% levels are same for A, B,C brands.

(1) Kruskal Wallis test for Low fat Yoghurt varieties for Na

```
Kruskal-Wallis Test on Na%
H0:all the treatment medians are same          Vs
H1:at least one is different from others

Low fat                 Ave
Yoghurt  N   Median     Rank      Z
1        3  0.01800     2.8  -1.68
2        3  0.01900     4.2  -0.65
3        3  0.32000     8.0   2.32
Overall  9              5.0

H = 5.76  DF = 2  P = 0.056
H = 5.80  DF = 2  P = 0.055  (adjusted for ties)
```

Interpretation:P value is 0.055and is > 0.05.So H0 is not rejected. So the median Na% levels are same for A, B,C brands.

(2) Kruskal Wallis test for Low fat Yoghurt varieties for K

```
Kruskal-Wallis Test on K%
H0:all the treatment medians are same          Vs
H1:at least one is different from others

Low fat                 Ave
Yoghurt  N   Median     Rank      Z
1        3  0.05540     3.7  -1.03
2        3  0.05000     3.3  -1.29
3        3  0.08590     8.0   2.32
Overall  9              5.0

H = 5.42  DF = 2  P = 0.066
```

Interpretation:P value is 0.066 and is > 0.05.So H0 is not rejected. So the median K% levels are same for A,B, C brands.

(3) Kruskal Wallis test for Low fat Yoghurt varieties for Ca

```
Kruskal-Wallis Test on Ca%
H0:all the treatment medians are same          Vs
H1:at least one is different from others

Low fat                 Ave
Yoghurt  N   Median     Rank      Z
1        3  0.9284      4.7  -0.26
2        3  0.9110      4.3  -0.52
3        3  0.9421      6.0   0.77
Overall  9              5.0

H = 0.62  DF = 2  P = 0.733
```

Interpretation:P value is 0.733 and is > 0.05.So H0 is not rejected. So the median Ca% levels are same for A, B,C brands.

(4) Kruskal Wallis test for Low fat Yoghurt varieties for Fe

```
Kruskal-Wallis Test on Fe%
H0:all the treatment medians are same          Vs
H1:at least one is different from others

Low fat                   Ave
Yoghurt  N     Median     Rank      Z
1        3  0.0003100     5.8   0.65
2        3  0.0001000     3.7  -1.03
3        3  0.0001900     5.5   0.39
Overall  9               5.0

H = 1.09  DF = 2  P = 0.580
H = 1.10  DF = 2  P = 0.578  (adjusted for ties)
```

Interpretation:P value is 0.578 and is > 0.05.So H0 is not rejected. So the median Fe% levels are same for A, B,C brands.

(1) Kruskal Wallis test for Simple Ice cream varieties for Na

```
 Kruskal-Wallis Test on Na%
H0:all the treatment medians are same          Vs
H1:at least one is different from others

Simple
ice                    Ave
cream     N   Median  Rank      Z
1         3  0.48100   8.0   2.32
2         3  0.02990   3.3  -1.29
3         3  0.02840   3.7  -1.03
Overall   9            5.0
H = 5.42  DF = 2  P = 0.066
```

Interpretation:P value is 0.066 and is > 0.05.So H0 is not rejected. So the median Na% levels are same for A,B,C brands.

(2) Kruskal Wallis test for Simple Ice cream varieties for K

```
Kruskal-Wallis Test on K%
H0:all the treatment medians are same          Vs
H1:at least one is different from others

Simple
ice                    Ave
cream     N   Median  Rank      Z
1         3  0.07400   7.3   1.81
2         3  0.03300   4.3  -0.52
3         3  0.03200   3.3  -1.29
Overall   9            5.0
H = 3.47  DF = 2  P = 0.177
```

Interpretation:P value is 0.177 and is > 0.05.So H0 is not rejected. So the median K% levels are same for A,B,C brands.

(3) Kruskal Wallis test for Simple Ice cream varieties for Ca

```
Kruskal-Wallis Test on Ca%
H0:all the treatment medians are same          Vs
H1:at least one is different from others

Simple
ice                    Ave
cream     N  Median  Rank      Z
1         3   1.100   6.0   0.77
2         3   1.081   4.7  -0.26
3         3   1.045   4.3  -0.52
Overall   9           5.0
H = 0.62  DF = 2  P = 0.733
```

Interpretation:P value is 0.733 and is > 0.05.So H0 is not rejected. So the median Ca% levels are same for A,B,C brands.

(4) Kruskal Wallis test for Simple Ice cream varieties for Fe

```
Kruskal-Wallis Test on Fe
H0:all the treatment medians are same          Vs
H1:at least one is different from others

Simple
ice                      Ave
cream     N     Median  Rank      Z
1         3  0.0001000   3.0  -1.55
2         3  0.0005800   5.7   0.52
3         3  0.0005700   6.3   1.03
Overall   9              5.0
H = 2.49  DF = 2  P = 0.288
```

Interpretation:P value is 0.288 and is > 0.05.So H0 is not rejected. So the median Fe% levels are same for A,B,C brands.

(1) Kruskal Wallis test for Complex Ice cream varieties for Na

```
Kruskal-Wallis Test on Na%
H0:all the treatment medians are same          Vs
H1:at least one is different from others

Complex
ice                    Ave
cream     N   Median   Rank      Z
1         3  0.04100    2.3  -1.53
2         3  0.04600    4.7   1.53
Overall   6             3.5

H = 2.33  DF = 1  P = 0.127
```

Interpretation:P value is 0.127 and is > 0.05.So H0 is not rejected. So the median Na% levels are same for A,B ,C brands.

(2)Kruskal Wallis test for Complex Ice cream varieties for K

```
Kruskal-Wallis Test on K%
H0:all the treatment medians are same          Vs
H1:at least one is different from others

Complex
ice                    Ave
cream     N  Median   Rank      Z
1         3  0.1354    3.3  -0.22
2         3  0.1412    3.7   0.22
Overall   6             3.5

H = 0.05  DF = 1  P = 0.827
```

Interpretation:P value is 0.827 and is > 0.05.So H0 is not rejected. So the median K% levels are same for A,B ,C brands.

(3) Kruskal Wallis test for Complex Ice cream varieties for Fe

```
Kruskal-Wallis Test on Fe%
H0:all the treatment medians are same          Vs
H1:at least one is different from others

Complex
ice                      Ave
cream     N     Median   Rank      Z
1         3  0.0005700    3.3  -0.22
2         3  0.0005800    3.7   0.22
Overall   6              3.5

H = 0.05  DF = 1  P = 0.827
H = 0.05  DF = 1  P = 0.825  (adjusted for ties)
```

Interpretation:P value is 0.825 and is > 0.05.So H0 is not rejected. So the median Fe% levels are same for A, B ,C brands.

(1) Kruskal Wallis test for Novelties varieties for Na

```
Kruskal-Wallis Test on Na%
H0:all the treatment medians are same          Vs
H1:at least one is different from others
                       Ave
Novelties  N   Median  Rank      Z
1          3  0.02270   3.7   0.22
2          3  0.02251   3.3  -0.22
Overall    6            3.5

H = 0.05  DF = 1  P = 0.827
```

Interpretation:P value is 0.827 and is > 0.05.So H0 is not rejected. So the median Na% levels are same for A,B,C brands.

(2) Kruskal Wallis test for Novelties varieties for K

```
 Kruskal-Wallis Test on K%
H0:all the treatment medians are same          Vs
H1:at least one is different from others

                      Ave
Novelties   N  Median  Rank      Z
1           3  0.1108   2.7  -1.09
2           3  0.1297   4.3   1.09
Overall     6           3.5
H = 1.19  DF = 1  P = 0.275
```

Interpretation:P value is 0.275 and is > 0.05.So H0 is not rejected. So the median K% levels are same for A, B, C brands.

(3) Kruskal Wallis test for Novelties varieties for Fe

```
Kruskal-Wallis Test on Fe%

H0:all the treatment medians are same          Vs
H1:at least one is different from others
                        Ave
Novelties   N     Median  Rank      Z
1           3  0.0002700   3.7   0.22
2           3  0.0002800   3.3  -0.22
Overall     6              3.5
H = 0.05  DF = 1  P = 0.827
```

Interpretation:P value is 0.827 and is > 0.05.So H0 is not rejected. So the median Fe% levels are same for A, B,C brands.

(1) Kruskal Wallis test for Ice cream categories to compare the distribution of Na% among the categories

```
Kruskal-Wallis Test on Na%
H0:all the median Na% values  are same among three ice cream categories
                        Vs
H1:at least one is different from others

Ice cream               Ave
categories  N   Median  Rank      Z
1           3  0.03140   3.3  -0.71
2           2  0.04225   5.0   0.77
3           2  0.04090   4.0   0.00
Overall     7            4.0

H = 0.71  DF = 2  P = 0.700
```

Interpretation:P value is 0.700 and is > 0.05.So H0 is not rejected. So the median Na% levels are same among the three ice cream categories.

(2) Kruskal Wallis test for Ice cream categories to compare the distribution of K% among the categories

```
Kruskal-Wallis Test on K%
H0:all the median Na% values  are same among three ice cream categories
                        Vs
H1:at least one is different from others

Ice cream               Ave
categories  N   Median  Rank      Z
1           3  0.04500   2.0  -2.12
2           2  0.13970   6.0   1.55
3           2  0.13460   5.0   0.77
Overall     7            4.0

H = 4.71  DF = 2  P = 0.095
```

Interpretation:P value is 0.095 and is > 0.05.So H0 is not rejected. So the median K% levels are same among the three ice cream categories.

(4) Kruskal Wallis test for Ice cream categories to compare the distribution of Fe% among the categories

```
Kruskal-Wallis Test on K%
H0:all the median Na% values  are same among three ice cream categories
                          Vs
H1:at least one is different from others

Kruskal-Wallis Test on Fe%

Ice cream                  Ave
categories  N     Median  Rank      Z
1           3  0.0006400   4.7   0.71
2           2  0.0005250   4.5   0.39
3           2  0.0003850   2.5  -1.16
Overall     7              4.0

H = 1.36  DF = 2  P = 0.507
```

Interpretation:P value is 0.507 and is > 0.05.So H0 is not rejected. So the median Fe% levels are same among the three ice cream categories.

(1) Kruskal Wallis test for Yoghurt categories to compare the distribution of Na% among the categories

```
Kruskal-Wallis Test on Na%
H0:all the median Na% values  are same among two Yoghurt categories
                          Vs
H1:at least one is different from others

Yoghurt                    Ave
categories  N   Median  Rank      Z
1           3  0.01800   2.7   0.45
2           1  0.01720   2.0  -0.45
Overall     4            2.5

H = 0.20  DF = 1  P = 0.655
```

Interpretation:P value is 0.655 and is > 0.05.So H0 is not rejected. So the median Na% levels are same among the two yoghurt categories.

(2) Kruskal Wallis test for Yoghurt categories to compare the distribution of K% among the categories

```
Kruskal-Wallis Test on K%
H0:all the median K% values  are same among two Yoghurt categories
                          Vs
H1:at least one is different from others

Yoghurt                    Ave
categories  N   Median  Rank      Z
1           3  0.05790   3.0   1.34
2           1  0.05400   1.0  -1.34
Overall     4            2.5

H = 1.80  DF = 1  P = 0.180
```

Interpretation:P value is 0.180 and is > 0.05.So H0 is not rejected. So the median K% levels are same among the two yoghurt categories.

(3) Kruskal Wallis test for Yoghurt categories to compare the distribution of Fe% among the categories

```
H0:all the median Fe% values  are same among two Yoghurt categories
                          Vs
H1:at least one is different from others

Kruskal-Wallis Test on Fe%

Yoghurt                      Ave
categories  N     Median   Rank      Z
1           3  0.0002100    2.7   0.45
2           1  0.0001900    2.0  -0.45
Overall     4               2.5

H = 0.20  DF = 1  P = 0.655
```

Interpretation:P value is 0.655 and is > 0.05.So H0 is not rejected. So the median Fe% levels are same among the two yoghurt categories.

APPENDIX IV

Statistical approach to make comparisons in between the selected dairy products

(1) Kruskal Wallis test to compare the nutritional value among the Yoghurt and Ice cream

H0: There is no difference in between the median Na% values among Yoghurt and Ice cream

Vs

H1: at least one is different from others

ice cream/yoghurt	N	Median	Rank	Z
1	4	0.01760	4.0	-1.51
2	7	0.04190	7.1	1.51
Overall	11		6.0	

H = 2.29 DF = 1 P = 0.131

Interpretation:P value is 0.131 and is > 0.05.So H0 is not rejected. So the median Na% levels are same among Ice cream and Yoghurt

(2) Kruskal Wallis test to compare the nutritional value among the Yoghurt and Ice cream

H0: There is no difference in between the median K% values among Yoghurt and Ice cream

Vs

H1: at least one is different from others

Kruskal-Wallis Test on K%

ice cream/yoghurt	N	Median	Ave Rank	Z
1	4	0.05645	4.5	-1.13
2	7	0.12870	6.9	1.13
Overall	11		6.0	

H = 1.29 DF = 1 P = 0.257

Interpretation:P value is 0.257and is > 0.05.So H0 is not rejected. So the median K% levels are same among Ice cream and Yoghurt

(3) Kruskal Wallis test to compare the nutritional value among the Yoghurt and Ice cream

H0: There is no difference in between the median Fe% values among Yoghurt and Ice cream

Vs

H1: at least one is different from others

Kruskal-Wallis Test on Fe%

ice cream/yoghurt	N	Median	Ave Rank	Z
1	4	0.0002000	4.8	-0.94
2	7	0.0004800	6.7	0.94
Overall	11		6.0	

H = 0.89 DF = 1 P = 0.345

Interpretation:P value is 0.345and is > 0.05.So H0 is not rejected. So the median Fe% levels are same among Ice cream and Yoghurt

(4) Kruskal Wallis test to compare the nutritional value among the Yoghurt and Curd

H0: There is no difference in between the median Na% values among Yoghurt and Curd

Vs

H1: at least one is different from others

Kruskal-Wallis Test on Na%

Curd/Yoghurt	N	Median	Ave Rank	Z
1	3	0.03800	5.0	1.06
2	4	0.01760	3.3	-1.06
Overall	7		4.0	

H = 1.13 DF = 1 P = 0.289

Interpretation:P value is 0.289and is > 0.05.So H0 is not rejected. So the median Na% levels are same among Curd and Yoghurt

(5) Kruskal Wallis test to compare the nutritional value among the Yoghurt and Curd

H0: There is no difference in between the median K% values among Yoghurt and Curd

Vs

H1: at least one is different from others

Kruskal-Wallis Test on K%

Curd/Yoghurt	N	Median	Ave Rank	Z
1	3	0.07200	5.0	1.06
2	4	0.05645	3.3	-1.06
Overall	7		4.0	

H = 1.13 DF = 1 P = 0.289

Interpretation:P value is 0.289and is > 0.05.So H0 is not rejected. So the median K% levels are same among Curd and Yoghurt

(6) Kruskal Wallis test to compare the nutritional value among the Yoghurt and Curd

H0: There is no difference in between the median Fe% values among Yoghurt and Curd

Vs

H1: at least one is different from others

Kruskal-Wallis Test on Fe%

Curd/Yoghurt	N	Median	Ave Rank	Z
1	3	0.0003000	5.0	1.06
2	4	0.0002000	3.3	-1.06
Overall	7		4.0	

H = 1.13 DF = 1 P = 0.289

Interpretation:P value is 0.289and is > 0.05.So H0 is not rejected. So the median Fe% levels are same among Curd and Yoghurt

(7) Kruskal Wallis test to compare the nutritional value among the Curd and Ice cream

H0: There is no difference in between the median Na% values among Yoghurt and Curd
Vs
H1: at least one is different from others

Kruskal-Wallis Test on Na%

Curd/Ice	N	Median	Ave Rank	Z
1	3	0.03800	4.3	-0.80
2	7	0.04190	6.0	0.80
Overall	10		5.5	

H = 0.64 DF = 1 P = 0.425

Interpretation:P value is 0.425 and is > 0.05.So H0 is not rejected. So the median Na% levels are same among Curd and Ice cream

(8) Kruskal Wallis test to compare the nutritional value among the Curd and Ice cream

H0: There is no difference in between the median K% values among Yoghurt and Curd
Vs
H1: at least one is different from others

Kruskal-Wallis Test on K%

Curd/Ice	N	Median	Ave Rank	Z
1	3	0.07200	4.0	-1.03
2	7	0.12870	6.1	1.03
Overall	10		5.5	

H = 1.05 DF = 1 P = 0.305

Interpretation:P value is 0.305 and is > 0.05.So H0 is not rejected. So the median K% levels are same among Curd and Ice cream

(9) Kruskal Wallis test to compare the nutritional value among the Curd and Ice cream

H0: There is no difference in between the median Fe% values among Yoghurt and Curd
Vs
H1: at least one is different from others

Kruskal-Wallis Test on Fe%

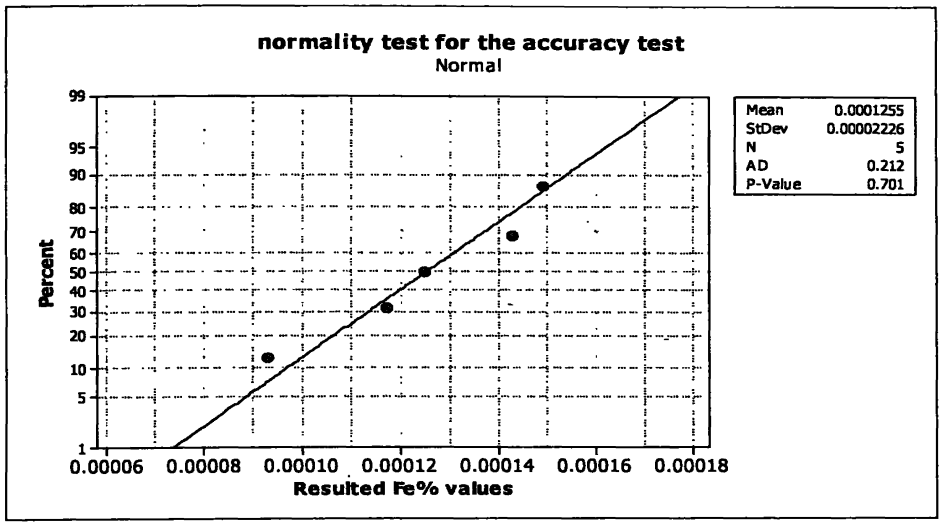
Curd/Ice	N	Median	Ave Rank	Z
1	3	3.00E-04	3.0	-1.71
2	7	4.80E-04	6.6	1.71
Overall	10		5.5	

H = 2.92 DF = 1 P = 0.087

Interpretation:P value is 0.087 and is > 0.05.So H0 is not rejected. So the median Fe% levels are same among Curd and Ice cream

APPENDIX V

The normality test for the accuracy test
H0:the data has normally distributed Vs
H1: the data has not normally distributed.



Interpretation: P value is 0.701 and is greater than 0.05. So H0 is not rejected. Therefore the data set obtained for the accuracy test has normally distributed.

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