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COMPARATIVE STUDY FOR THE DETERMINATION OF METALS IN MILK SAMPLES USING FLAME-AAS AND EDTA COMPLEXOMETRIC TITRATION

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Abstract: A comparative study was carried out for the determination of calcium and magnesium in 5 different brands of milk (Haleeb, Milk Pak, Olpers, Dairy Queen and Nurpur). After dry ashing and treatment with concentrated nitric acid, calcium and magnesium were determined by Flame Atomic Absorption Spectrometry (FAAS) and complexometric titration with EDTA. Erichrome Black-T and patton and reeder indicator were used for calcium and magnesium determination by complexometric titration method. The values obtained for Ca and Mg were found to be within WHO limts

Keywords: calcium; magnesium; complexometric titration; flame atomic absorption spectrometry.

Introduction

Milk is a complex material consisting of several components, which have a significant role even though present in low concentrations. Increased awareness of the influence of diet on human health has prompted producers to produce food of higher quality which should be rich in nutrients and vitamins. Good quality measurements are essential to control, maintain products and process quality, in manufacturing, trade and research. This is important, since raw materials for food production are becoming poor in essential minerals.[1]

Milk and dairy products have been recognized worldwide as a good source of wholesome nutrition for the human body. Milk contains a large variety of essential nutrients for the development and maintenance of a salutary life.[2] It is an outstanding source of calcium and phosphorus, and can supply moderate amounts of magnesium, zinc, iron and copper.[3,4] These essential metals are of interest to determine the adequate daily intake by an organism. However, due to an increase in environmental pollution, it is necessary to determine and monitor the level of toxic metals in milk, such as cadmium and lead, as they may have adverse effects on human health. Calcium contributes to structural functions in bones and teeth alongwith regulating many biological functions.

More recently, focus on calcium has centered on its role in preventing osteoporosis.[5] Magnesium is the fourth most abundant cation in the human body after sodium, potassium and calcium. Most of the magnesium in the body is deposited in bones.[6] The understanding of milk magnesium composition is important for nutritional management during early life.[7] Copper has great biological importance in milk whereby higher copper contents in milk contribute towards acceleration of lipid oxidation. Recently the action of copper in several important diseases has been reported.[8,9] The function of nickel is not completely understood as an essential element in the human body but its importance in the human body needs to be further researched. This work combines analysis using atomic absorption spectroscopy for the determination of metals (Ca, Mg, Fe, Ni, Cu and Cd) in 5 different milk brands

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(Haleeb, Milk Pak, Olpers, Dairy Queen, and Nurpur) and a complexometric titration with EDTA for determination of Ca and Mg. A comparison was made to measure the levels of Ca and Mg in milk and standard deviation. Values were calculated for these metals (Ca, Mg, Fe, Ni, Cu and Cd) and compared to WHO limits.[10]

Materials and Methods

Standard Solutions:-

For analysis of the samples by AAS, standard solutions of Ca, Mg, Fe, Ni, Cu and Cd of analytical grade (Merck) were used and the appropriate dilutions were made accordingly.

Preparation of Sample Solutions:-

5 different milk brands of Haleeb, Milk Pak, Olpers, Dairy Queen and Nurpur were purchased from local markets of Lahore to prepare the sample solutions.

Drying of Sample:-

A 100 ml covered porcelein crucible was used in which, 5 ml of milk sample was evaporated to dryness in a drying oven (Binder) at 100°C. This allowed for powdered milk to be obtained which is easily preserved. The shelf life of powdered milk is longer than liquid milk and so there is no need to refrigerate it due to its low moisture content.

Ashing of Sample:-

The dried powdered milk was then placed in a porcelein crucible and transferred to a graphite furnace (Box-type Resistance Furnace Model SX-5-12). The temperature was increased slowly at a maximum rate of 50°C/hr to 450°C and heating was continued until the ash turned white. The sample was cooled overnight and then ground using a pestle and mortar into finely divided powdered form.

Preparation of Solution:-

To powdered white ash, 50ml of deionised water and 5 ml of concentrated nitric acid was added. The solution was gently stirred and heated to dissolve the ash contents completely after which the solution was filtered to remove the undissolved particles. The solution was then transferred into a 100 ml volumetric flask and the volume was made upto the mark using deionised water.

Reagents and Solutions:-

All reagents used were of analytical grade (Merck). Deionised water was used for the preparation of all solutions. Triethanol amine and ethyl alcohol (Analytical Grade) were used for the preparation of organic reagent Eriochrome Black-T, as an indicator, and Ethylene diamine tetreacetic acid (EDTA), was used as a complexing agent. An alkaline buffer of ammonium chloride-ammonia (pH 10) was used to maintain pH and concentrated HNO_3 was used for the digestion of organic matter. Patton and reeder indicator was used in the complexometric titration of calcium along with KOH for pH adjustment.

Preparation of 0.01M EDTA Solution:-

0.01 M EDTA solution was prepared by dissolving 3.7724g EDTA (Na2H2Y.2H2O) in 500 ml deionised water with constant stirring for 15 min in a 1L volumetric flask. When the solid crystals of EDTA were completely dissolved, the volume was made upto the mark.

Preparation of Buffer Solution:-

An alkaline buffer of ammonium chloride-ammonia (pH 10) was prepared by dissolving 17.5g of ammonium chloride (NH4Cl) in 50 ml deionised water in a 250 ml volumetric flask. To this, 142 ml of concentrated liquid NH3 was added and the volume was made

upto the mark to form a buffer of pH 10.

Preparation of Eriochrome Black T Indicator:-

For preparation of this indicator, 0.2g of EDT (3-hydroxy-4-[(1-hydroxy-2-naphthyl) azo-7-nitro-1-naphthalene-sulphonic acid, sodium salt) was dissolved in 15 ml of triethanolamine with the addition of 5 ml of absolute ethanol to obtain fresh indicator. The solution was filtered and stored between 0 and 5°C.

Instrumentation:-

The absorbance of the solution was measured with atomic absorption spectrophotometer (Hitachi-Polarised Zeeman Atomic Absorption Spectrophotometer Model Z-500). Electric Balance (Scout Pro OHAUS) was also used for weighing purposes.

General Procedure:-

Sample Analysis by Atomic Absorption for Ca, Mg, Fe, Cu, Ni and Cd

A Hitachi-Polarized Zeeman Atomic Absorption Spectrophotometer, Z-5000 was used for the determination of Ca, Mg, Fe, Cu, Ni and Cd in different brands of milk The samples were run in triplicate. Table 1 and 2 shows the working parameters for each element analysed.

Table 1: Working parameters – standard conditions for Hitachi-Polarised (Zeeman Atomic Absorption Spectrophotometer (Model Z-5000).

Metal	Wavelength (nm)	Slit width (nm)	Time constant (s)	Lamp current (mA)
Ca	422.8	1.3	1.0	9.0
Mg	285.3	1.3	1.0	9.0
Fe	248.3	1.3	1.0	9.0
Cu	324.8	1.3	1.0	9.0
Ni	232	0.2	1.0	12
Cd	228.8	1.3	1.0	9.0

Table 2: Parameters of AAS analysis

Metal	Atomizer	Flame type	Fuel flow (1/min)	Oxidant (kPa)	Burner height (mm)
Ca	Standard	Air- C_2H_2	2.2	160	7.5
Mg	Standard	Air- C_2H_2	2.2	160	7.5
Fe	Standard	Air- C_2H_2	2.2	160	7.5
Cu	Standard	Air- C_2H_2	2.2	160	7.5
Ni	Standard	Air- C_2H_2	2.2	160	7.5
Cd	Standard	Air- C_2H_2	2.2	160	7.5

Calcium and Magnesium Determination in Milk by Complexometric Titration

For calcium determination, 20ml of sample was taken in a conical flask and 2-3 pellets of KOH were added. After shaking the solution 1 g of Patton and reeder indicator (calcon 3-carboxylic acid) was added and the sample was titrated against 0.01M EDTA solution until a color change from wine red to blue appeared.

For magnesium, 20ml of sample was taken in a conical flask and 2-3ml of buffer solution was added followed by 4-5 drops of Eriochrome Black T indicator. The sample was then titrated against 0.01M EDTA solution until the color changed from wine red to blue.

RESULTS

Calcium

The concentration of calcium in different brands is 2.50, 2.02, 2.23, 2.80 and 2.67 as determined by atomic absorption spectroscopy. Values by EDTA titration are 4.8, 4, 5, 4 and 4. The values of standard deviation by atomic absorption and EDTA titration are 0.28 and 0.43.

Magnesium

The concentration of magnesium in different brands of milk is 1.64, 1.29, 1.52, 1.11 and 1.15 as determined by atomic absorption. Values by EDTA titration are 3.39, 3, 4.4, 3.9 and 2.50 respectively. The standard deviation values by atomic absorption and EDTA titration are 0.20 and 0.62.

Iron

The concentration of iron in different brands of milk is 0.56, 0.59, 0.62, 0.58 and 0.56 as determined by atomic absorption. The standard deviation is 0.02.

Copper

The concentration of copper in different brands of milk is 0.21, 0.18, 0.14, 0.13 and 0.13 as determined by atomic absorption. The standard deviation is 0.03.

Nickel

The concentration of nickel in different brands of milk is 0.08, 0.08, 0.08, 0.08 and 0.08 as determined by atomic absorption. The standard deviation is 0.28.

Cadmium

The concentration of cadmium in different brands of milk is 0.08, 0.07, 0.07, 0.07 and 0.07 as determined by atomic absorption. The standard deviation is 0.26.

Table 3: Analysis of metals by AAS analysis

Elements	Milk Brands				
	Haleeb (1)	Milkpak (2)	Olpers (3)	Dairy Queen (4)	Nurpur (5)
Ca	2.50	2.02	2.23	2.80	2.61
Mg	1.64	1.29	1.52	1.11	1.15
Fe	0.56	0.59	0.62	0.58	0.56
Cu	0.21	0.18	0.14	0.13	0.13
Ni	0.08	0.08	0.08	0.08	0.08
Cd	0.08	0.07	0.07	0.07	0.07

Table 4: Analysis of metals by complexometric method

Elements	Milk Brands				
	Haleeb (1)	**Milkpak (2)**	**Olpers (3)**	**Dairy Queen (4)**	**Nurpur (5)**
Ca	4.8	4	5	4	4
Mg	3.39	3.3	4.4	3.9	2.5

Discussion

The method used for the digestion of organic matter is dry ashing or digestion. There are two types of digestions, wet digestion and dry digestion. Dry digestion is preferred over wet digestion because wet digestion is time consuming and requires an excess of strong acid which interferes with the analyzers. Use of nitric acid is preferred over the other acids because nitric acid is an acceptable matrix for both flame and electrothermal analysis. Sulphuric acid, perchloric acid and hydrochloric acid interfere in the analysis of some metals and all provide a poor matrix.

Using AAS it was found that Ca is highest in sample 4 and lowest in sample 2. Analysing by complexometric titration showed higher concentration of Ca in sample 3. The values for magnesium are 1.64, 1.29, 1.52, 1.11 and 1.15 as determined by atomic absorption spectrometry. The higher value for magnesium is found in sample number 1 for atomic absorption and for complexometry the higher value is found for sample number 3. The values of calcium and magnesium by titration are 4.8, 4, 5, 4, 4 and 3.39, 3, 4.4, 3.24 and 2.50 in the samples 1, 2, 3, 4 and 5 respectively. Both the values as determined by atomic absorption spectroscopy and complexometric titration are within the WHO limits. The values obtained by titration are almost double the values by atomic absorption spectrometry because titration is an indirect method and is not as precise as atomic absorption spectrometry.

Other than calcium and magnesium values for iron, copper, nickel and cadmium were also found. Values for iron in sample number 1, 2, 3, 4 and 5 are 0.56, 0.59, 0.62, 0.58, 0.56. Values for copper in sample number 1, 2, 3, 4 and 5 are 0.21, 0.18, 0.14, 0.13 and 0.13 respectively. Values for nickel in sample number 1, 2, 3, 4 and 5 was found to be the same (0.08). Values for cadmium in sample number 1, 2, 3, 4 and 5 are 0.08, 0.07, 0.07, 0.07 and 0.07 respectively. Iron is present in highest concentration in sample number 3 and lowest in samples 1 and 5. Higher concentration of copper has been found in sample number 1 and lower in samples 4 and 5. The value for nickel is the same in all the samples. For cadmium all the samples have the same value other than sample number 1 which is higher than all. As these elements are present in very low concentrations so these elements cannot be found by complexometric titration with EDTA. The most adequate method for determining these metals is by Atomic Absorption Spectroscopy.

The values for the standard deviation for calcium and magnesium by Atomic Absorption Spectroscopy and complexometric titration by EDTA are 0.28, 0.20, 0.43 and 0.62 respectively. The values for standard deviation for iron, copper, cadmium and nickel are 0.02, 0.03, 0.28 and 0.26 respectively.

A graph is plotted between the different elements found in milk i.e. calcium, magnesium, iron, copper, nickel and cadmium. Calcium is found to be present in higher concentrations and cadmium is

found to be present in lower concentrations. Higher concentrations of calcium are desirable as milk is a rich source of calcium.

In the case of heavy metals, iron is present in higher concentrations and cadmium is present in lower concentrations. All these concentrations are within the WHO limits. The values for all the minerals indicate that all the brands of milk are very well processed and are drinkable.

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