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Comparative Determination of Calcium in Some Food Substances Using EDTA Titrimetric and Atomic Emission Spectroscopic Methods

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Abstract: *This research is aimed at comparing the concentration of Calcium (Ca) in some food substances using EDTA titrimetric method and atomic emission spectroscopic (AES) methods. The food substances are Milk (4 different animal milk samples), yoghurt (5 different yoghurt samples), vegetables (5 different green leafy vegetables samples), meat (5 different animal meat samples), fruits (5 different types of fruits samples), fish (4 different types of fish samples), and cheese (3 different types of cheese samples). The statistical analysis result of this study indicated significant differences exist in concentration of yoghurt samples (t=2.9328, p=0.019), fruit samples (t=2.4851, p=0.038) and fish samples (t=4.2908, p=0.005) determined using EDTA titrimetric and atomic emission spectroscopy (AES). The result of linear regression analysis indicated that strong positive relationship (r=0.8576) exist between the EDTA titrimetric method and atomic emission spectroscopic methods for the determination of calcium in all the seven (7) groups of food samples analyzed. This is an indication that both method can be use satisfactorily for determination of calcium in food samples but atomic emission spectroscopy (AES) is more effective.*

Keyword: *Calcium, EDTA titration, atomic emission spectroscopy (AES), food substances.*

INTRODUCTION

Foods in the form of carbohydrates, fats and protein supply the human body with the required energy for metabolic processes. Foods also provide the body with substances such as amino acids, vitamins and minerals, all of which are required for growth , maintenance of cells and tissues (Lawani *et. al*., 2014). Calcium (Ca) is an essential mineral that play vital roles in human nutrition. This mineral is commonly found in various food substances, including dairy products, leafy greens, nuts, and grains. Monitoring the levels of calcium in these food sources is crucial for ensuring their nutritional value and understanding their potential health effects. Food substances consist essentially of carbohydrate (grains, legumes, wheat, potatoes, cassava, maize, wheat, flour yams), fat (vegetable oils, nuts, seeds, fish), protein (egg, fish, beans, lentils, beef, yoghurt, cheese, nuts), minerals (cereals, bread, meat, fish, milk, dairy) and vitamins (nuts, fruit and vegetables, milk and dairy foods) used in the body of an organism to sustain growth and vital processes and to furnish energy (Wangare, 2023). Various food substances contribute to a well-rounded and

nutritious diet when consumed in appropriate portions and as part of a balanced eating plan. Among these food substances include:

a) Milk: a nutrient-rich liquid produced by mammals, primarily cows. It is a good source of calcium, vitamin D, and protein. Milk is often consumed as a beverage or used in cooking and baking.
b) Yoghurt (Yogurt): a dairy product made by fermenting milk with beneficial bacteria. It is rich in probiotics, which are beneficial for gut health. Yogurt is available in various flavors and is commonly eaten on its own, added to smoothies, or used as a topping.
c) Vegetables: edible plants that are rich in vitamins, minerals, and dietary fiber. They come in various forms, including leafy greens (spinach, lettuce), root vegetables (carrots, potatoes), cruciferous vegetables (broccoli, cauliflower), and more. Vegetables are an important part of a balanced diet.
d) Meat: the flesh of animals, typically from livestock such as cows, pigs, chickens, and sheep. It is a significant source of protein and essential nutrients like iron and B vitamins. Common types of meat include beef, pork, poultry, and lamb.
e) Fruit: naturally sweet and colorful foods that are rich in vitamins, minerals, and antioxidants. They come in various forms, such as berries (strawberries, blueberries), citrus fruits (oranges, lemons), tropical fruits (pineapple, mango), and more. Fruits are a healthy choice for snacks and desserts.
f) Fish: a protein-rich food that provides omega-3 fatty acids, which are beneficial for heart and brain health. Different types of fish include salmon, tuna, cod, and trout. Fish can be baked, grilled, or fried and is a popular choice for many cuisines.
g) Cheese: a dairy product made from curdled milk. It comes in a wide variety of textures, flavors, and types, such as cheddar, mozzarella, and Swiss. Cheese is often used as a topping, ingredient in dishes, or enjoyed on its own.

In the realm of analytical chemistry, the accurate quantification of essential minerals in the above food substances holds paramount significance due to their vital roles in human health and nutrition. Among the essential minerals, calcium stand out as pivotal element contributing to various physiological processes (Weaver, *et. al*., 2014)

Calcium is a mineral most often associated with healthy bones and teeth, although it also plays an important role in blood clotting, helping muscles to contract, and regulating normal heart rhythms and nerve functions. About 99% of the body's calcium is stored in bones, and the remaining 1% is found in blood, muscle, and other tissues (Harvard, 2023). Calcium, the most abundant mineral in the body, is found in some foods, added to others, present in some medicines (such as antacids), and available as a dietary supplement. Calcium makes up much of the structure of bones and teeth and allows normal bodily movement by keeping tissue rigid, strong, and flexible. The small ionized pool of calcium in the circulatory system, extracellular fluid, and various tissues mediates blood vessel contraction and dilation, muscle function, blood clotting, nerve transmission, and hormonal secretion (Heaney, 2010). Calcium from foods and dietary supplements is absorbed by both active transport and by passive diffusion across the intestinal mucosa (Weaver and Heaney, 2014). Active transport is responsible for most absorption when calcium intakes are lower, and passive diffusion accounts for an increasing proportion of calcium absorption as intakes rise.

Calcium is a necessary trace element in human body. The calcium needed by human body per day is about 120 to 1400 mg. About 80% of this come from food and about 20% are provided by drinking water containing calcium. The lack of calcium element is bound to cause harm to human health. Calcium is not only the basic raw material for bone development and directly influences on stature but also has other important physiological functions in body (Gao, 2005). These functions play an important role in maintaining the health of the body and ensuring the smooth development of normal growth. Calcium can promote the activities of some enzymes in the body, regulate the activity of enzymes, and participate in the activities of the nerves, muscles, and the release of neurotransmitters (Li and Zhai, 2020). Calcium is also important in regulation of the secretion of hormones, blood coagulation, cell adhesion, and muscle contraction. Calcium also has the functions to regulate heart rhythm, reduce cardiovascular permeability, control inflammation and edema, and maintain acid-base balance. In daily life, if calcium intake is

insufficient, the human body will suffer from physiological calcium overdraft, resulting in a decrease in blood calcium levels. Calcium is an essential element for humans, and is also responsible for 'water hardness', a water quality parameter frequently tested in industrial plants and municipal water treatment facilities (Filik *et. al*., 2011). Calcium is biologically required for numerous functions, such as building and maintaining the bones and teeth, blood clotting, transmitting of the nerve impulses and regulating heart's rhythm. More recently, focus on calcium has centered on its role in preventing osteoporosis. Calcium deficiency leads to osteoporosis, hyperosteogeny, rickets in children, spasm of the hand and foot, hypertension, kidney stones, colon cancer, dementia, and other diseases. However, excessive calcium intake can also give rise to adverse effects, such as increasing the risk of kidney stones and affecting the absorption of other metals elements, such as iron and magnesium (Li and Zhai, 2020).

Various analytical techniques have been developed to quantitatively analyze minerals in food samples, each with its own set of advantages and limitations. The concentrations of some minerals require more sensitive instrumental analytical techniques such as flame atomic absorption spectrometry (FAAS), inductively coupled plasma (ICP) emission spectrometry, capillary electrophoresis, ion chromatography, and spectrophotometry, though the most widely used one is AAS (Filik *et al*., 2011). Analytical procedures currently employed for the determination of calcium are EDTA titration, precipitation, and weighing of calcium oxalate, re-dissolving precipitated calcium oxalate and titrating with permanganate, flame photometry, and atomic absorption spectrophotometry (Lee, 2017). These techniques can offer distinct approaches to determine the concentration of calcium ions in food substances and can provide valuable insights into their comparative effectiveness.

Atomic Emission Spectroscopy (AES) is a sophisticated and modern analytical technique that offers exceptional sensitivity and selectivity in elemental analysis. AES is particularly advantageous for its ability to simultaneously analyze multiple elements in a sample and its low sample consumption. (Liu. *et. al.,* 2020).

EDTA titrimetric is a classical and widely used method in analytical chemistry for the determination of metal ions, including calcium and magnesium. This technique relies on the formation of stable complexes between the metal ions and EDTA, a chelating agent. The endpoint of the titration is detected using indicators or potentiometric methods, enabling accurate quantification of metal ion concentration. EDTA titrimetric is known for its reliability, precision, and cost-effectiveness, making it a popular choice in routine analyses.

Traditional analytical methods, such as titrimetric, have been used for decades to quantify calcium in food samples. Ethylenediaminetetraacetic acid (EDTA) titration is a widely employed method for determining metal ions due to its chelating properties. Atomic Emission Spectroscopy (AES) can provide qualitative and quantitative information about the elemental composition of a sample. This research will help in choosing best and less expensive method between the two. Therefore, this study aims at Comparative Determination of Calcium (Ca) in Food Substances using EDTA Titrimetric Analysis and Atomic Emission Spectroscopy (AES).

MATERIALS AND METHODS

In the preparation of reagents, chemicals of analytical grade purity and deionized water were used. All glass wares were washed with liquid reagent and rinsed with deionized water before drying in an oven at 105ºC. All weighing were carried out using analytical weighing balance model FA2004 and the essential elements were analyzed using Atomic Emission Spectroscopy (AES) Machine Box Scientific Model 230.

Sampling and sample preparations.

Thirty one (31) samples of food substances from seven (7) food groups viz: Milk (cow milk, goat milk, sheep milk and carmel milk.), Yoghurt (san yoghurt, namhto yoghurt, basako yoghurt, ashna yoghurt, and hanan yoghurt.), and Vegetables (spinach, bitter leaf, moringa leaf, pumpkin leaf, and lettuce.), Meat (cow

meat, chicken meat, pigeon meat, duck meat and ram meat.), Fruits (orange, papaya, dry date, banana, and strawberry), Fish (canned fish, stock fish sardine, and tilapia fish) and Cheese (fromage fondu cheese, carmel cheese, and cow cheese.) were purchased from local market and retail store in Zaria local government, Kaduna state.

For the vegetables samples the leaves were removed from their stems, washed properly with tap water and rinsed with deionized water and dried for three days under the shade to avoid direct contact with sun, after three days the samples were then placed in oven over night at 125^{o}C until they were completely dried and were made to powdered form using stainless steel motor and pestle, the dried samples were sieved with 2 mm stainless steel sieve, and transferred into air-tight plastic container and labeled appropriately.

For fruits samples, the seed of the fruits were removed completely and discarded and the fruits were washed properly with tap water and rinsed with deionized water and cut them in into smaller pieces and dried for three days under the shade to avoid direct contact with sun, after three days the samples were then placed in oven over night at 125^{o}C until they were completely dried and were made to powdered form using stainless steel motor and pestle, the dried powdered samples were transferred into air-tight plastic container and labeled appropriately.

For the meat samples, all the animals were slaughtered and collect some part of their meat and washed properly with tap water and rinsed with deionized water and cut into smaller pieces and dried for three days under the shade to avoid direct contact with sun, after three days the samples were then placed in oven over night at 125^{o}C until they were completely dried and were made to powdered form using stainless steel motor and pestle, the dried powdered samples were transferred into air-tight plastic container and labeled appropriately.

The same process applied to fish and cheese samples.

The liquid samples (Yoghurt and Milk) were also dried in the shade for five days and placed in an oven for four hours at 140^{o}C, and the sample was then ashed in the muffle furnace to burn all the organic matter in the sample and then transferred the ashed sample into container and labeled appropriately.

Preparation of standard solution.

0.01M EDTA solution was prepared by dissolving 3.7724g EDTA ($Na_2H_2Y.2H_2O$) in 500cm^3 deionized water with constant stirring for 15 min in a 1L volumetric flask. Crystals of EDTA were completely dissolved, the volume was made up to the mark (Hussain *et al.,* 2010).

An alkaline buffer of ammonium chloride-ammonia (pH 10) was prepared by dissolving 17.5g of ammonium chloride (NH4Cl) in 50cm^3 deionized water in a 250cm^3 volumetric flask. To 142cm^3 of concentrated liquid NH_3 was added and the volume was made to the mark (Hussain *et al.,* 2010).

Exactly 0.2g of Eriochrome Black T indicator was dissolved in 15cm^3 of concentrated ammonia solution and 5cm^3 absolute ethanol, the solution was allowed to completely dissolve for two hours and transfer into indicator container (Hussain *et al.,* 2010).

Ashing.

The ashing was carried out as reported by (Hussain *et al.,* 2010) the dried milk and yoghurt samples was placed in clean porcelain crucibles and transferred to muffle furnace, the temperature was increased slowly at a maximum rate of 50^{o}C/hour to 450^{o}C and the heating was continue until the ash turn white. The sample was cold overnight and grind using stainless steel mortar and pestle, the ashed samples were then transferred into air-tight plastic container and labeled appropriately.

Digestion.

1.00g of the powdered sample was accurately weighed in already weighed, clean and dried cm^3 Pyrex beaker. Where $8cm^3$ of the concentrated sulfuric acid and $10cm^3$ of concentrated nitric acid were added. The beaker was then placed on a hot plate and warmed cautiously and until the reaction subsided.

To avoid charring a $2cm^3$ of aliquot of concentrated nitric acid was constantly added at any time the solution began to darken. At this point the solution was allowed to cool and diluted with $10cm^3$ distilled water and boiled to fuming. At this point, the persistence of color(s) of the solution are cleared by the addition of about $4cm^3$ of hydrogen peroxide with drops of nitric acid. The solution was heated to fuming state each time hydrogen peroxide was added until the residue was colorless or no further reduction of pale yellow color was obtained. Then the solution was made up to mark in $100cm^3$ volumetric flask the mixtures were filtered using filter paper no. 1. The filtrate was then transferred into a $50cm^3$ volumetric flask and made to the mark with deionized water, transferred into $50cm^3$ sample bottles (Lawani. *et al.,* 2014).

Triplicate analysis was carried out. An aliquot of each digested solution was used for the determination of calcium (Ca) by atomic emission spectroscopy (AES) Method and another aliquot by EDTA titrimetric method.

EDTA titration.

A $25.0cm^3$ aliquot of each digested sample was pippeted into a cm^3 beaker and 1M NaOH solution was added to adjust the pH to 12-13 two drops of eriochrome black T indicator was added and immediately titrated against a 0.01M EDTA solution to the end point. (Lawani. *et al*., 2014). For every determination and evaluation, a standard curve for mass of calcium (mg) versus amount of EDTA (millimoles) was recorded.

Statistical analysis.

The statistical analysis of all the data was conducted using SPSS software (version 20.0). Statistical t test was used to determine the significance of the concentrations determined from the two methods of analysis in the samples at $p < 0.05$ of the significance level. Linear regression analysis was also conducted to find the relation between the two methods of analysis.

RESULT AND DISCUSSION

The results for the comparative determination of calcium in some food substances using EDTA titrimetric and atomic emission spectroscopic methods are presented below:

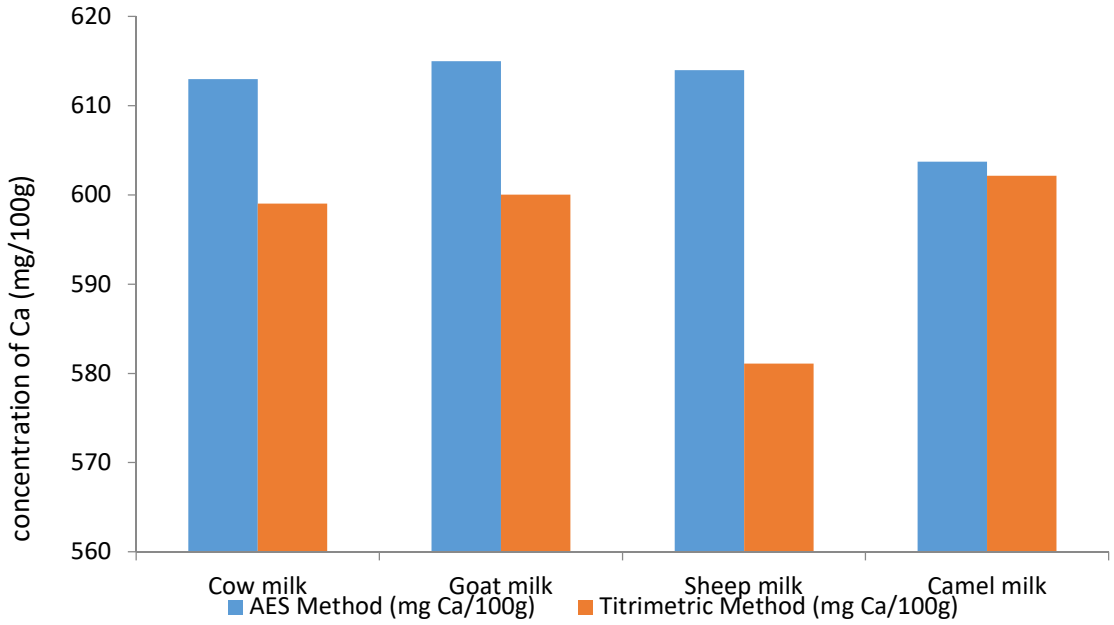

Fig 1 Mean concentration of Ca in the milk samples analyzed using AES and titrimetric methods

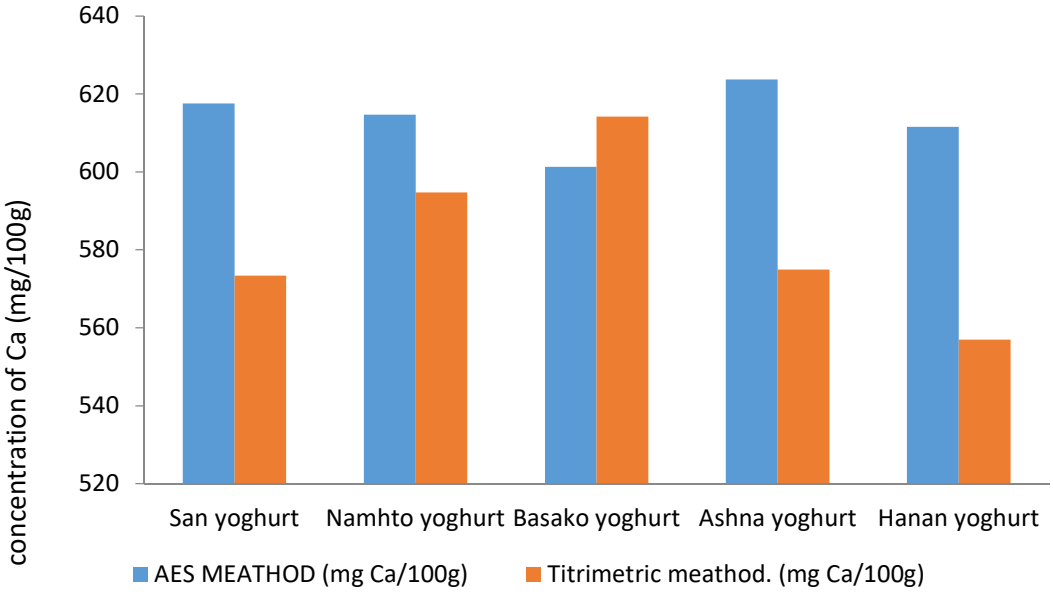

Fig 2: Mean concentration of Ca in the yoghurt samples analyzed using AES and titrimetric methods.

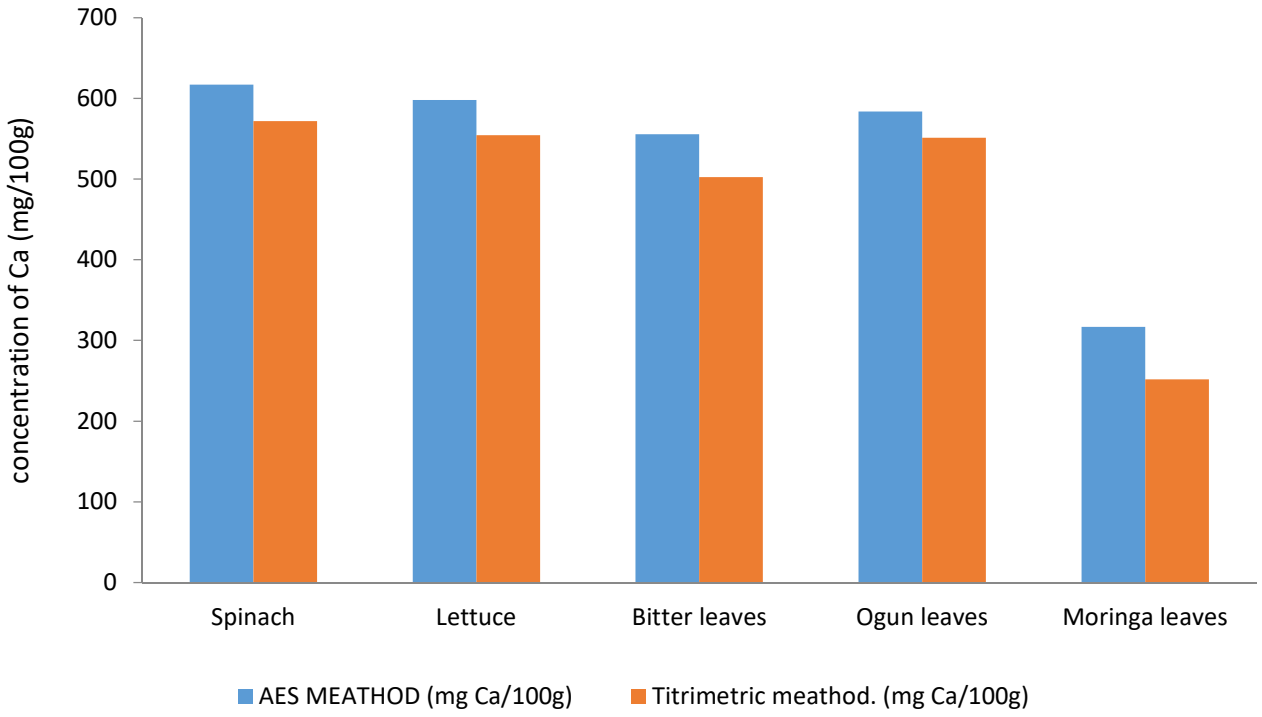

Fig 3: Mean concentration of Ca in the vegetable samples analyzed using AES and titrimetric methods.

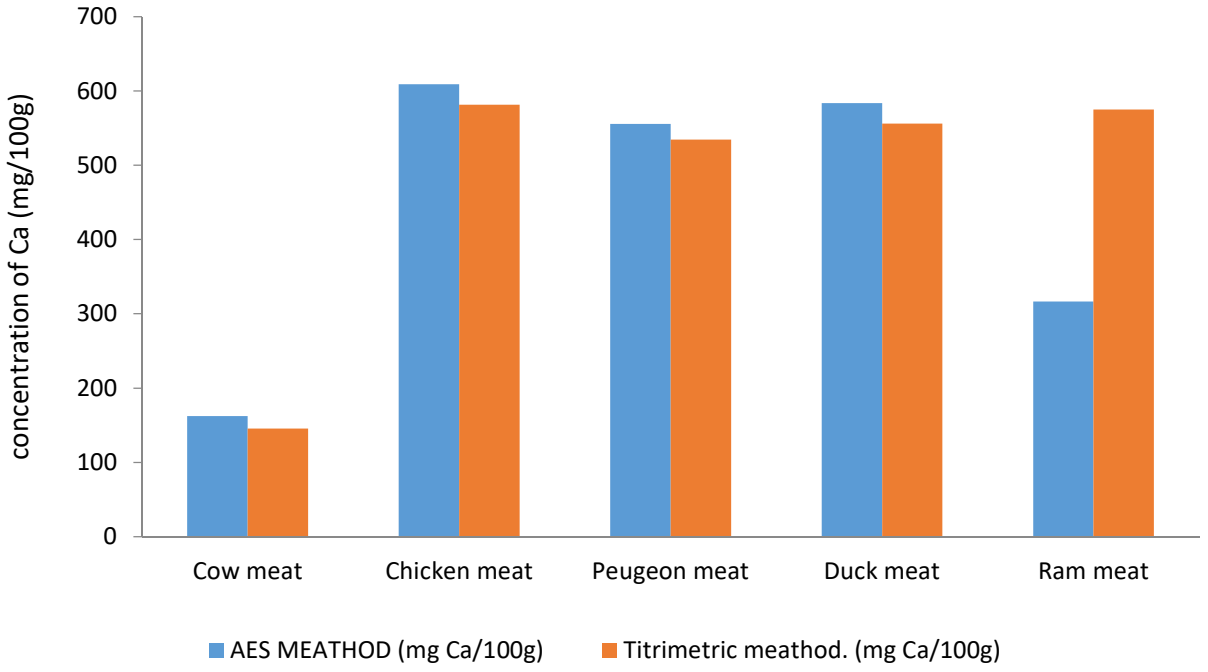

Fig 4: Mean concentration of Ca in the meat samples analyzed using AES and titrimetric methods.

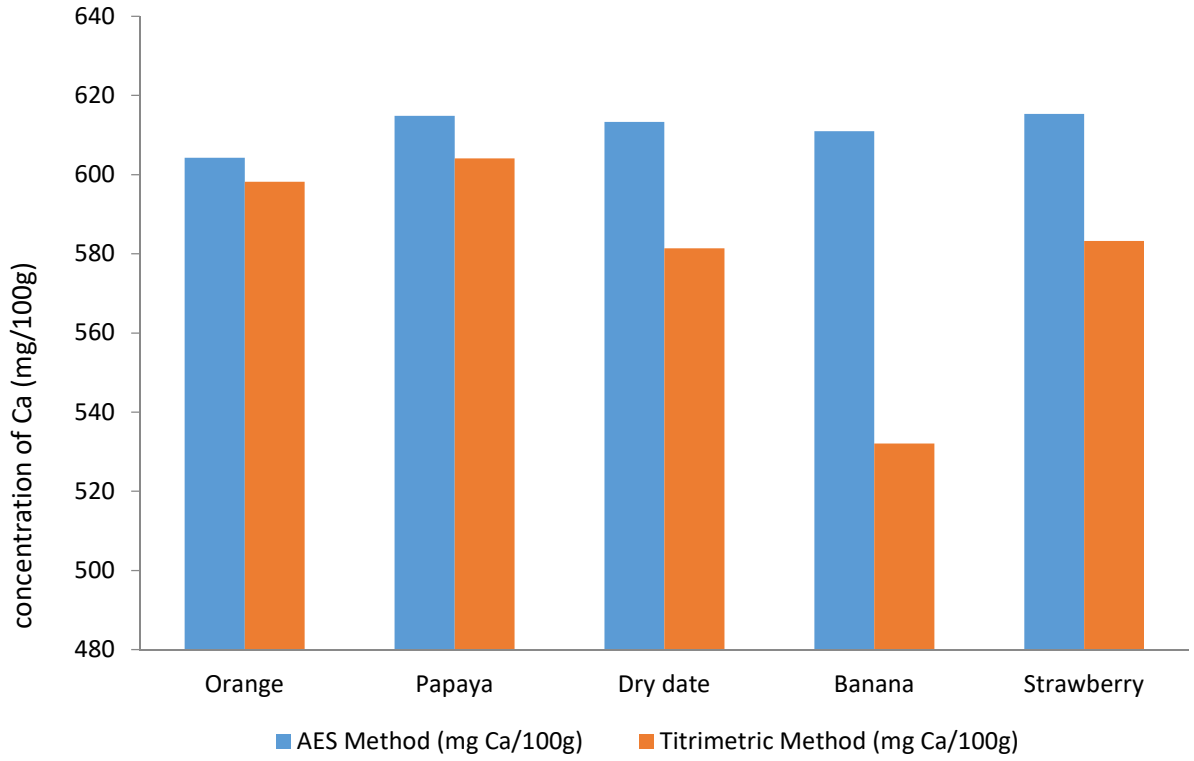

Fig 5: Mean concentration of Ca in the fruit samples analyzed using AES and titrimetric methods

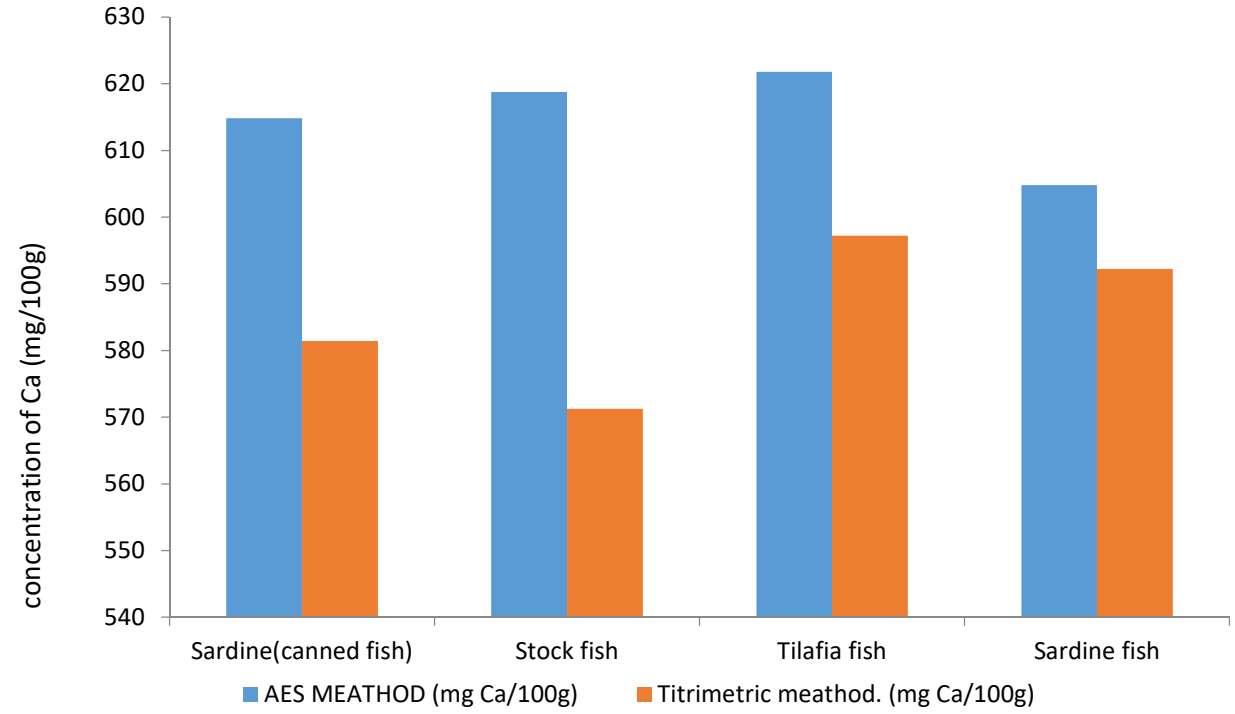

Fig 6: Mean concentration of Ca in the fish samples analyzed using AES and titrimetric methods.

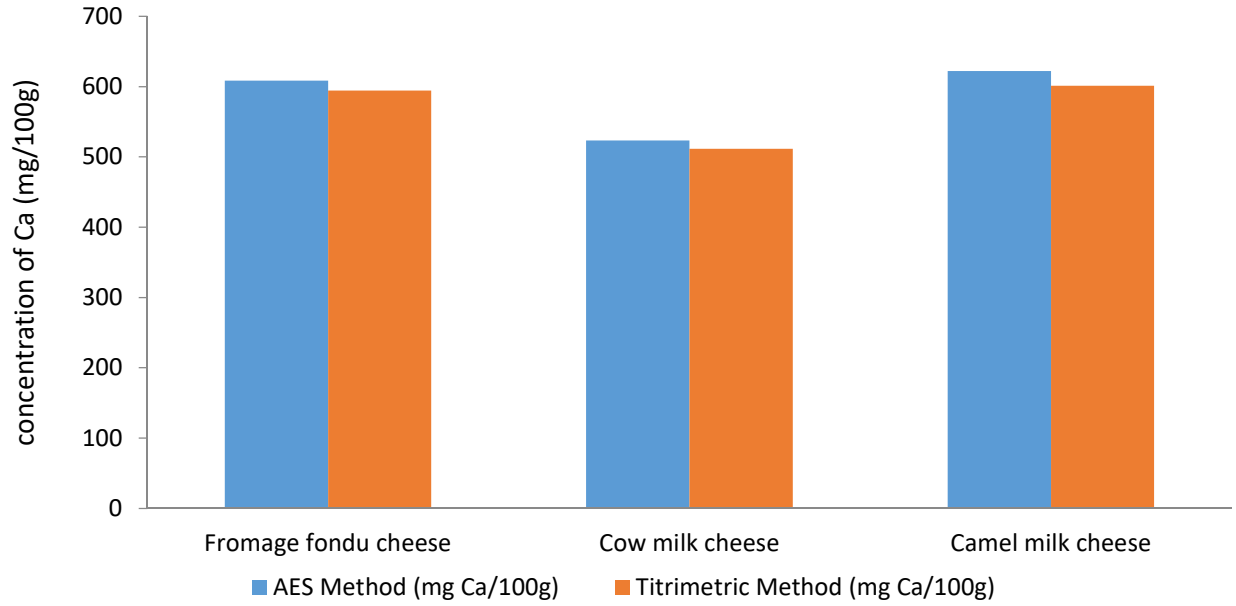

Fig 7: Mean concentration of Ca in the cheese samples analyzed using AES and titrimetric methods.

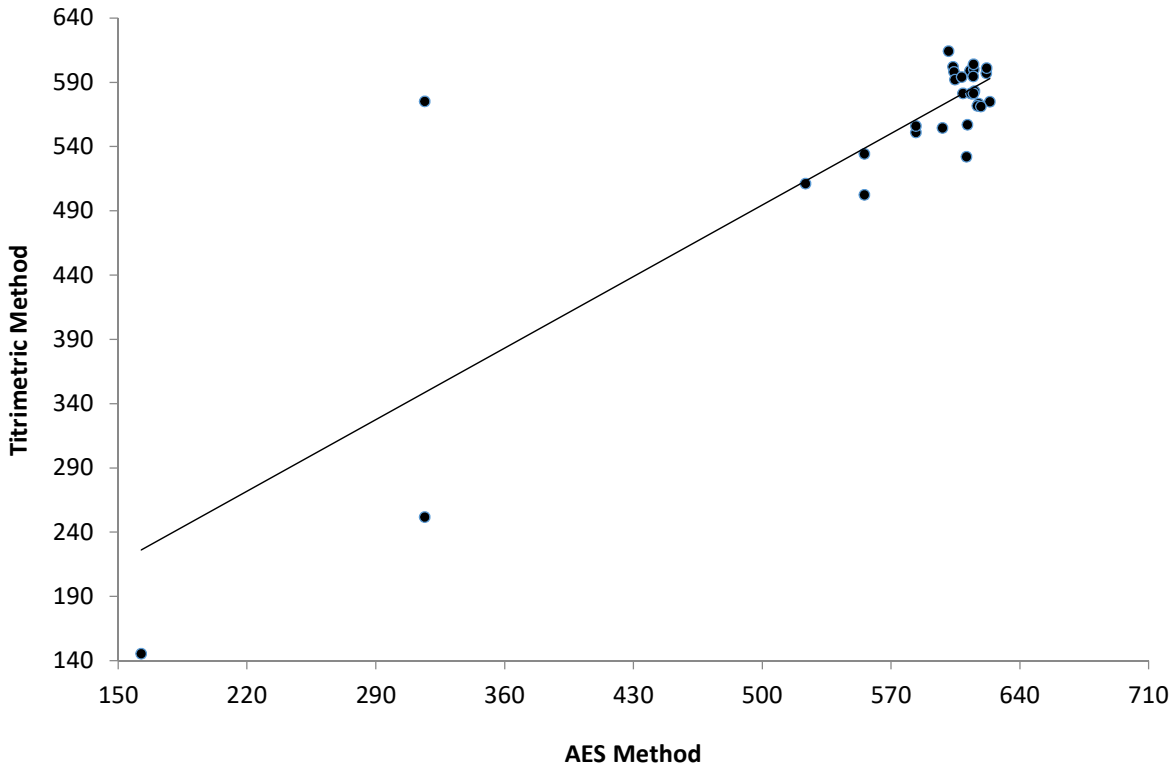

Fig 8: Linear regression analysis showing the relationship in concentrations of calcium determined in the seven (7) food samples using AES and titrimetric methods.

Discussion

Concentration of calcium in Milk samples

Cow milk, goat milk, and sheep milk were found to have the highest calcium concentrations determined using atomic emission spectroscopy (AES) having 612.96mg/100g, 614.97mg/100g and 613.97mg/100g respectively, and Carmel milk was found to be lower than them having 603.71mg/100g. On the other hand the concentrations of calcium determined using EDTA titrimetric method in Carmel milk, goat milk, and cow milk were highest with 609.14mg/100g, 600.01mg/100g and 599.01mg/100g respectively while sheep milk has lowest value of 581.11mg/100g. (Hussain *et al.,* 2010) from Lahore India reported a research titled; comparative study of the determination of metals in milk samples using flam AAS EDTA complexometric. the result of the findings are similar to the findings of this research, (TEE *et al.* 1989) also reported a research on comparative determination of calcium in different animals milk and the result 604mg/100g, 587mg/100g and 600.45mg/100g which similar to this research, (Kapadnis 2015) also reported the result of research titled; magnesium and calcium estimation from fresh milk samples in nashik region by a simple volumetric method, the values of the concentrations of calcium in the research are; 982.06mg/100g, 876.00mg/100g, 788.10mg/100g and 838.82mg/100g. The values are high than the one in this research. The recommended dietary allowances of calcium in adult is1300mg, this implies that 200 – 250g of milk one can achieve the recommended dietary intake.

Concentration of calcium in yoghurt samples

Yoghurt is a dairy product made by fermenting milk with beneficial bacteria, the result of the concentrations of calcium in the yoghurt samples determined using atomic emission spectroscopy (AES) revealed almost all the yoghurt samples have the same calcium value with little variation in ashna yoghurt having highest value among all the yoghurt samples623.74mg/100g. While on the EDTA titrimetric method Ashna yoghurt also has the highest value followed by nahmto yoghurt, basako yoghurt and san yoghurt. 614.20mg/100g, 612.66mg/100g, 601.28mg/100g and 600.22mg/100g respectively, this result indicate that atomic emission spectroscopy (AES) method provide highest concentration of calcium compared to titrimetric method of analysis. (Hussain *et al.,* 2010) and (Lawani et al., 2014) reported similar result of the analysis.

Concentration of calcium in vegetable samples

Vegetables are the edible parts of plants that are consumed wholly or in part, raw or cooked. The result of calcium concentrations in vegetables indicated little variation from the following samples spinach has highest concentration with 616.92mg/100g, lettuce 597.97mg/100g, bitter leaf 555.52mg/100g, pumpkin leaf 583.57mg/100g and moringa with lowest value of 316.63mg/100g concentrations determined using atomic emission spectroscopy (AES) while the values obtained using EDTA titrimetric method were lowest compare to those obtained using (AES) methods. (Lawani et al., 2014) from alhikmah university Ilorin Nigeria reported the result of research on titrimetric determination of calcium content in some staples foodstuffs in north-central Nigeria among the samples are some vegetables which have lower value than the one in this research, this might be as a result of different chemicals content of the soils on which they were cultivated.

(Sion. *et al.,* 1989) reported the concentration of calcium in some vegetables, spinach have 480mg/100g, moringa leaves have 307mg/100g, the values the differences in calcium concentration determined in the same food stuff and different sampling areas and values reported by other researchers from different regions of the world could be attributed to several factors. This include differences in species cultivated, farming methods and methods of chemical analysis (Nordeide *et al.,* 1996 and Mayer, 1997).

Concentration of calcium in Meat samples

Meat is the flesh of animal, typically from livestock such as cows, chicken, sheep etc. it's a significant source of protein and essential nutrients like calcium, iron, magnesium etc. the general result of concentrations of calcium determined in meat samples using atomic emission spectroscopy and EDTA showed little variation between the two methods, Cow meat has lowest value of calcium from both methods while chicken meat, pigeon meat, and duck meat have highest calcium value for both methods of analysis (Corrao. *et al.,* 2020) reported the concentrations of calcium in meat samples in his research titled; titrimetric determination of calcium in mechanically separated poultry and beef as beef have 170mg/100g, poultries have 518.23mg/100gand 339.01mg/100g. (A.C Germs and H. Steunenberg. 2000) also reported the concentrations of calcium in research titled; estimation of calcium in mechanically deboned poultry meat by oxidimetry and atomic absorption spectroscopy the values are 2.36g/kg, 2.5g/kg and 0.40g/kg. This indicate that poultry meat contains high concentrations of calcium than beef meat.

Concentration of calcium in Fruit samples

Fruits are naturally sweet and colorful foods that are rich in vitamins and minerals. The result of calcium concentrations in fruits using atomic emission spectroscopy (AES) revealed highest values of calcium in the fruits samples whereas EDTA titrimetric method revealed lowest value. This finding is in line with study by (Lawani *et al* 2014) which reported the concentration of the banana 375mg/100g, and orange 297mg/100g. (Sion *et al.,* 1989) also reported the value of dry date 420mg/100g. This variation might be as a result of different location where the fruits are cultivated. (Sa'eed and Abdullahi. 2012) reported research titled; calcium and iron levels in some fruits and vegetables commonly consumed in Kano metropolis, Nigeria. The calcium values of the fruits in the research are in line with the values of this research.

Concentration of calcium in Fish samples

Fish is a protein-rich food that provide essential elements to the body such as calcium and magnesium. The result obtained for the analysis indicate that tilapia fish, canned fish and stock fish have the highest values of calcium determined using atomic emission spectroscopy (AES) method as 621.77mg/100g, 614.81mg/100g and 618.81mg/100g respectively. The EDTA titrimetric method gives low values of 592.24mg/100g, 581.42mg/100g and 571.24mg/100g respectively, (Sion *et al.,* 1989) reported a significantly lower result of research on concentration of calcium values of fish 547mg/100g, 418mg/100g, 450.1mg/100g and 390.6mg/100g respectively this indicate that the calcium value of fish depends on variety of the fishes. The result also indicate that fish is a good and reliable source of calcium because the values were found to be closer to the recommended daily allowance (RDA) by FOA/WHO.

Concentration of calcium in Cheese samples

Cheese is dairy product made from curdled milk. It's one of the best source of calcium. The result of the study of calcium determined in cheese samples using atomic emission spectroscopy (AES) are fromage fondu cheese has 608.30mg/100g, cow milk has 523.52mg/100g and Carmel cheese has 621.92mg/100g. The Carmel cheese has the highest value followed by fromage fondu cheese, while the EDTA titrimetric method reported results lower than AES method. The result are in line with finding of (Nathalie Ramillard and Michel Britten 2011) research titled quantitative determination of micellar calcium in milk and cheese using acid-base titration reported the concentrations of two different cheese 390.1mg/100g and 559.7mg/100g which is almost similar to the result of this work. The bioavailability of calcium from cheeses meets can be deduced from the fact that, for example 150 – 180g of cheese meets the recommended daily intake for calcium. The nutritional values of cheese also depends on the fat and the type of cheese (Hrkovic-porobija *et al.,* 2021).

CONCLUSION

Result of this study established that Atomic Emission Spectroscopy (AES) and EDTA titrimetric methods gave significantly differences in the determination of calcium (Ca) oncentrations for a wide variety of foods (yoghurts, fruits and fish). Both methods showed strong positive relationship in concentrations of calcium (Ca) determined in the seven (7) food groups samples. And both methods showed an excellent recovery values. Both methods show weak positive relationship. Either methods can therefore be used satisfactorily for the analysis of calcium (Ca) in food substances but atomic emission spectroscopy (AES) is more effective method.

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