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Research paper

Comparative study of some macro & micro elements in milk samples from Abuja, Nigeria using microwave plasma atomic emission spectroscopy (MP-AES) and flame atomic absorption spectroscopy (F-AAS) methods

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Abstract

A comparative study was carried out for the determination of calcium, magnesium, iron and zinc contents in two brands of powdered milk samples, using the Microwave Plasma Atomic Emission Spectroscopy (MP-AES) and Flame Atomic Absorption spectroscopy (F-AAS) methods. The powdered milk samples were collected and placed in a muffle furnace at 550°C for 6 hours, and the ash contents obtained was used for the sample preparation. The percentage recoveries for the spikes were as follows: for MP-AES determination; the recoveries for the milk samples were within the range (116.9 – 163.1%) for calcium; (51.8 – 78.8%) for magnesium; (110.9 – 125.4%) for iron; (90.7 – 92.9%) for zinc. For FAAS determination; the recoveries for the milk samples were within the range (51.1 – 83.6%) for calcium; (52.2 – 61.9%) for magnesium; (77.7 – 84.8%) for iron; (46.5 – 71.7%) for zinc. The percentage recoveries showed a better recovery with MP-AES than the F-AAS method. The analysis of variance conducted using One way ANOVA method shows that there is no significant difference between the two methods used for the analysis, as the P-values (calculated probability) obtained were higher than 0.05. The values obtained for Ca, Mg, Fe and Zn content shows that only Ca was found to be within WHO/FAO standard, while Mg, Fe and Zn were found to be below the WHO/FAO standard. It is highly recommended that a 100g of any of the powdered milk used for the research work be consumed per day in order to meet up with the RDA per day, or other sources of these elements to be considered.

Keywords: *Flame Atomic Absorption Spectroscopy (F-AAS); Macro and Micro Elements; Microwave Plasma Atomic Emission Spectroscopy (MP-AES); Percentage recovery.*

1. Introduction

Milk is a white liquid produced by the mammary gland of mammals. It is a primary source of nutrients for young mammals before they are able to digest other types of food (Islam et al., 2014). Milk provides the energy required for human activities and nutrients needed for building up the human body (Yoo et al., 2013). Milk and other dairy products have been recognized all over the world for a long time, not only for their sensory properties, but also for their beneficial influence on human health (Steijns, 2001). Sources of milk from domesticated animal include not only of cattle but also of sheep, goat and buffalo (Imran et al., 2008).

Milk is known as an excellent source of calcium, and it can supply moderate amounts of magnesium, smaller amounts of zinc and very small amounts of iron and copper (Pennington et al., 1995).

India is the largest producer of milk throughout the world. In the world, there are more than 6 billion consumers of milk and milk products (Michael et al., 1991).

Over 750 million people live within dairy farming households. Nigeria is the largest producer of cow milk in West Africa, and third in Africa (Michael et al., 1991).

Milk contains water, carbohydrate, fats, protein, enzyme, vitamins, organic acids, and minerals like phosphorus, potassium and calcium (Imran et al., 2008).

Milk is one of the most important food commodities in the world, because it's well known as a substantial source of several nutrients such as proteins, enzymes, fats, vitamins and essential elements (also known as minerals), and hence plays a key role during all phases of human life. However, the chemical and physical composition of milk is among the important public health issues for milk consumers (Mansouri-Najandi & Sharifi, 2013). For this reason, it is essential to make sure that milk consumers are paying for quality and accurate dairy products. This assurance can only be guaranteed as a result of research studies like this.

2. Materials and methods

2.1. Reagents and solutions

All reagents used were of analytical grade. Distilled water was used for the preparation of all solutions. All glassware used were properly cleaned by first washing with detergent and water, and then rinsed severally with distilled water and dried.

2.2. Sampling

A total of sixteen (16) samples of powdered milk (8 samples each of Peak and Dano), were randomly purchased from three markets in Abuja (Gwarimpa, Wuse, and Gwagwalada). The samples were labeled and taken to the laboratory to prepare the working solutions.

2.3. Preparation of unspiked milk samples solutions for MPAES and AAS analysis.

A 10g of each of the two milk samples (Peak and Dano) was weighed into three different crucible and placed in a furnace at 550ºC for 6 hours. The ashed samples were brought out, cooled and then digested with 20ml of 0.1M $HNO_3$and heated until the metals are completely dissolved. The digests were cooled and decanted into 100ml volumetric flasks. The digestion kits were then rinsed with distilled water and rinses from the digestion kits poured into the volumetric flasks until they were made up to mark (Nabil, 2011). The resulting mixtures were carefully transferred into samples bottles labeled 1, 2 and 3 respectively for each brands of milk samples, which was then used for analysis

2.4. Preparation of spiked milk samples solution for MPAES and AAS analysis.

The procedure for the unspiked samples was repeated up to ashing. The cooled ashes were then spiked with 20 ml each of 100mg/dm^3calcium and magnesium and 2ml each of 100mg/dm^3iron and zinc mixed standard solutions.
The mixtures were digested with 20 ml of 0.1M HNO_3 and heated until the metals were completely dissolved. The digests were then cooled, and decanted into 100ml volumetric flasks. The digestion kits were rinsed with distilled water, and rinses from the digestion kits were poured into the volumetric flasks until they were made up to mark (Nabil, 2011). The resulting mixtures were carefully transferred into sample bottles labeled 4, 5 and 6 respectively for each of the brands of milk samples.

3. Results and discussions

The results of the mineral element composition of the different brands of powdered milk studied are summarized in Table 1.

Table 1: The Composition of Mineral Elements of Powdered Milk Samples Using MP-AES & AAS (Mg/Kg)

	MPAES		AAS	
	Sample1	Sample2	Sample1	Sample2
Ca (mg/kg)	828.96±0.91	821.71±1.56	853.15±5.32	839.74±5.34
Ca Spike	855.64±4.21	851.39±2.39	867.31±6.29	851.30±5.83
R (%)	134.06±21.44	148.39±18.78	70.91±10.50	57.73±9.31
Mg (mg/kg)	11.97±0.34	10.42±0.45	26.35±0.92	21.29±0.57
Mg Spike	22.64±0.22	22.35±0.21	37.81±2.43	35.79±0.81
R (%)	53.36±1.10	59.63±2.31	57.23±8.63	72.46±5.23
Zn (mg/kg)	0.213±0.08	0.365±0.04	0.081±0.04	0.0825±0.02
Zn Spike	1.410±0.01	1.361±0.04	1.908±0.01	1.935±0.03
R (%)	59.86±3.82	50.19±4.28	91.37±0.67	92.65±0.19
Fe (mg/kg)	0.08±0.02	0.054±0.01	0.01±0.07	0.01±0.01
Fe Spike	1.71±0.09	1.73±0.03	2.30±0.09	2.51±0.08
R (%)	79.99±3.71	83.93±1.00	115.05±4.60	125.08±3.78

Key: Sample 1 Peak milk, Sample 2 Dano milk.

Table 1 shows the results obtained from the analysis of sample 1 and 2 to determine the Ca, Mg, Fe, and Zn contents using the Microwave Plasma Atomic emission spectrometer (MP-AES) and Flame Atomic Absorption spectroscopy (F-AAS) methods respectively. The calcium content level in sample 1 with an average mean of 828.96 mg/100g (8,289.6 mg/kg) was found to be slightly more in concentration than that of sample 2 which has an average mean of 821.70 mg/100g (8,217mg/kg). This shows that both samples are rich source of calcium and are within the standard set by the WHO/FAO (812.6 mg/100g), and the Recommended Dietary Allowance (RDA) per day (800 – 1200 mg), according to FNB (1989).
Comparing the calcium concentration levels of sample 1 and 2 with that reported by Lawal et al. (2015) 9,000-15,000, and also Akpanyung (2006) 11,108 mg/kg shows that it was lower.
The average concentration of magnesium in sample 1 and 2 was found to be 26.35 mg/100g (263.5 mg/kg), and 21.29 mg/100g (212.90 mg/kg) respectively.
These are significantly lower than that reported by Lawal et al. (2015) 920-1000 mg/ kg and Akpanyung (2006) 1000 mg/kg, and also lower than the RDA per day (270 - 400 mg) according to FNB (1989).
The average iron concentration of sample 1 which is 0.0014 mg/100g (0.014 mg/kg) was found to be higher than that of sample 2 with 0.015 mg/100g (0.15 mg/kg) concentration respectively. The concentration levels of both sample 1 and 2 were found to be significantly lower than that reported by Lawal et al. (2015) 82-161 mg/kg, Salah et al. (2013) 20.41 ppm, and Perween et al. (2011) 4.33 mg/kg, and also lower than the RDA per day (11 - 15 mg) according to FNB (1989) respectively.
The average zinc concentration of sample 1 was found to be 0.081 mg/100g (0.81 mg/kg) and that of sample 2 was 0.083 mg/100g (0.83 mg/kg). These are significantly lower than what was reported by Lawal et al. (2015) 65.79 mg/kg, Akpanyung (2006) 126.1 mg/kg, and Semaghiul et al. (2008) 3.24 ppm respectively, and were also below the standard set by WHO/FAO (11.1 mg/100g), according to FNB (1989).

The use of MP-AES showed that analysis of variance significance test was higher than 0.05, indicating no significant difference in comparing the two milk sample used in the study.

The analyses of Ca, Mg, Fe, and Zn using the Flame Atomic Absorption spectrometer (FAAS) in table 1 revealed that the average mean concentration of calcium in sample 1 was 853.15 mg/100g (8,531.5 mg/kg), and that of sample 2 was 839.74 mg/100g (8,397.4 mg/kg) respectively.

This shows that both samples are rich source of calcium, and are within the standard set by the WHO/FAO (812.6 mg/100g), according to FNB (1989).

Comparing the calcium concentration levels of sample 1 and 2 shows they are lower than that reported by Lawal et al. (2015) 9,000-15,000mg/ kg, and also Akpanyung (2006) 11,108 mg/kg.

The average concentration of magnesium in sample 1 and 2 was found to be 11.97 mg/100g (119.7 mg/kg), and 10.42 mg/100g (104.20 mg/kg) respectively. These are also significantly lower than that reported by Lawal et al. (2015) 920-1000 mg/ kg and Akpanyung (2006) 1000 mg/kg, and also lower than the RDA per day (270 – 400 mg) and below the standard set by the WHO/FAO (275.0 mg/100g), according to FNB (1989) respectively.

The average Fe concentration of sample 1 which is 0.079 mg/100g (0.79 mg/kg) was found to be higher than that of sample 2 which is 0.054 mg/100g (0.54 mg/kg) respectively.

The concentration levels of both sample 1 and 2 were found to be significantly lower than that reported by Lawal et al. (2015) 82-161 mg/kg, Salah et al. (2013) 20.41 ppm, Perween et al. (2011) 4.33 mg/kg and also lower than the RDA per day (11 - 15 mg) and below the standard set by the WHO/FAO (11.1 mg/100g), according to FNB (1989) respectively.

The average zinc concentration of sample 1 was 0.80 mg/100g (8.0mg/kg) and that of sample 2 0.83 mg/100g (8.3 mg/kg) respectively. These concentrations are significantly lower than what was reported by Lawal et al.(2015) 65.79 mg/kg, Akpanyung (2006) 126.1 mg/kg, and also lower than the RDA per day (11 - 15 mg) and below the standard set by the WHO/FAO (8.90mg/100g), according to FNB (1989), but higher than that reported by Semaghiul et al. (2008) 3.24 ppm. The statistical analysis shows no significant difference as P-value tends to be greater than 0.05.

Table 2: Comparison of the Average Percentage Recoveries of Spiked / Unspiked Milk Samples Using the Two Instrumental Methods

	MP-AES		AAS	
	Sample1	Sample2	Sample1	Sample2
Ca	828.96	821.70	853.15	839.74
Ca Spike	856.56	851.39	867.31	851.30
AV.R	139.68	148.43	70.82	57.83
Mg	11.97	10.42	26.35	21.29
Mg Spike	22.64	22.35	37.81	35.79
AV.R	53.37	59.65	57.17	72.40
Zn	0.80	0.37	0.081	0.083
Zn Spike	1.412	1.36	1.909	1.934
AV.R	70.62	50.33	91.38	92.59
Fe	0.079	0.054	0.001	0.015
Fe Spike	1.706	1.733	2.302	2.512
AV.R	81.32	83.95	115.05	124.89

KEY: AV.R – Average Percentage Recovery, P> 0.05 (Calc. Probability).

Table 2 shows the comparison between the average percentage recoveries of calcium and magnesium levels in both sample 1 and 2, using the two instrumental methods. The table shows that the average percentage recovery of Ca content in sample 1 was 70.82% and 57.83% in sample 2, while that of Mg content in sample 1 was found to be 57.17% and 72.40% in sample 2, from the AAS analysis. These were found to be slightly within the same range with that reported by Deborah (2012) for Ca (50.0 - 83.3) % and Mg (48.5 - 83.5) %.

From the above table, the recoveries of the spikes for the AAS analysis of all the elements are less than 100%, when compared to the MP-AES analysis. This may be due to some interferences which may have led to the reduction in the number of atoms formed in the flame.

These interferences may have been as a result of ionization of the atoms by the flame or the presence of some chemical species (matrix of the sample) in the samples, which leads to the formation of some compounds that could not be dissociated by the flame.

Although different ways of addressing the effect of non-spectral interferences in the determination of metals using flame atomic absorption spectroscopy have been employed, but they do not completely suppress the effects of these interferes.

For examples, the use of cold flame (that is air-acetylene flame) and standard addition method may address the effect of ionization and to an extent, matrix effect, but leaving behind the effect of chemical interferences.

The use of hot flame (nitrous oxide-acetylene flame), standard addition method, and ionization buffer may address the effect of chemical interference, and to an extent, matrix effect and ionization effect, but due to the fact that these interferences are not completely addressed, the systematic error encountered in the use of FAAS for the determination of calcium, magnesium, iron and zinc is relatively great.

The MP-AES analysis gave better percentage recoveries, ranging from values very close or greater than 100%, as its' microwave induced plasma eliminates most of the interferences possibly encountered in FAAS. Also its' high level of sensitivity, superior linear dynamic range, detection limits and analysis speed makes it a better method of analysis for these metals than the conventional flame atomic absorption spectroscopy.

The analysis of variance (ANOVA) between the MP-AES and AAS shows that at $P > 0.05$, no significant difference between these two methods used in analysis of the mineral elements in the milk samples.

The results obtained from the two methods of analysis shows that only calcium contents were found to be within the standard set by WHO/FAO, in the two brands of milk samples.

The contents of these minerals when compared with previous works done on milk samples showed a drastic reduction in the contents over the years, which signals that some of these diary and diary product manufacturers may have significantly reduced the fortification of their powdered milk during production which may have affected these mineral contents, since they are not routinely monitored. An average Nigerian does not consume 100g of powdered milk at any particular instant in a day (Akpanyung, 2006).

Rather about three (3) teaspoons of milk (approx. 25 g) are added to a cup of beverage before drinking (Akpanyung, 2006).

If one is to consider any of these powdered milks (Peak and Dano) as his/her only source of these nutrients, it means he/she will need to take about 3 to 4 glass of milk per day, as Mg, Fe, and Zn contents were not sufficient enough in the milk samples studied.

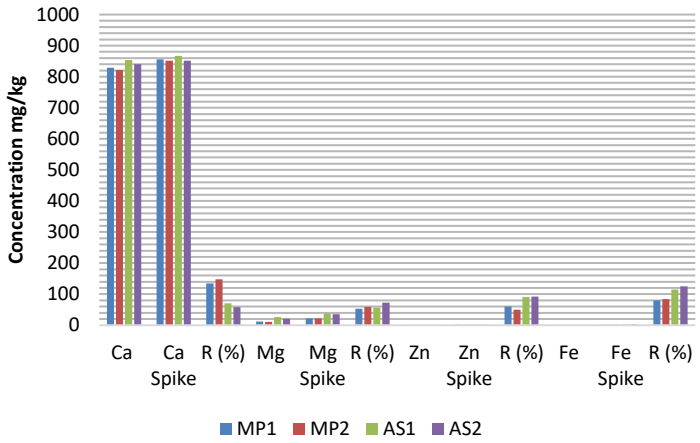

Fig. 1: Chart Showing the Comparison of Elements in Milk Samples Using the Two Instrumental Methods.

Key: MP1 – MP-AES Sample 1 (Peak); MP2 - MP-AES Sample 2 (Dano) AS1– AAS Sample 1 (Peak); AS2 - AAS Sample 2 (Dano); R (%) - Recovery

Figure 1 shows a chart comparing the mean concentration of the unspiked and spiked elements in the two brands of milk samples, using the two instrumental methods.
The concentration of calcium for both unspiked and spiked samples were enormously higher than that of magnesium, and that of iron and zinc which were in trace quantities. The chart shows both MP-AES and FAAS are excellent methods for the determination of Ca and other elements in the milk samples.

4. Conclusion

From this research work, the results obtained in chapter four showed that the Ca, Mg, Fe, and Zn contents in the milk samples were successfully determined at 95% confidence level using two instrumental and two classical methods, and the two brands of powdered milk were found to be very rich in calcium, as they are within the WHO/FAO standards. On the other hand, magnesium, iron and zinc contents were found to be below the limit set by the WHO/FAO.
The analysis of variance conducted using One way ANOVA method shows that there is no significant difference between the two methods used for the analysis, as the P-values obtained in comparison were greater than 0.05.
In terms of percentage recoveries, the MP-AES method of analysis was found to have higher percentage recoveries, and therefore a better instrumental method for the determination of Ca, Mg, Fe and Zn in milk samples than the conventional FAAS method.

5. Conflicts of Interest

The authors declare no conflicts of interest.

6. Authors' contribution statement

Conceptualization and supervision: Faruruwa, M. D. and Mohammed, Y.; Experiment and Manuscript writing: Salawu, S. J.: Project design and supervision: Salawu, S. J., Faruruwa, M. D. and Mohammed, Y., Manuscript review: Salawu, S. J. and Abubakar S.; Manuscript writing and editing: Salawu, S. J., Abubakar S.

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