SCIENCE AND TECHNOLOGY - Research Journal - Volume 3 - 1999
University of Mauritius, Réduit, Mauritius.

EVALUATION OF ATOMIC ABSORPTION SPECTROPHOTOMETRY (ASHING, NON-ASHING) AND TITRIMETRY FOR CALCIUM DETERMINATION IN SELECTED FOODS

by

V. BISSESSUR[1], D. GOBURDHUN[1]* *and* A. RUGGOO[2]

[1]Department of Agricultural and Food Science and [2]Department of Agricultural Production and Systems, Faculty of Agriculture, University of Mauritius, Réduit, Mauritius

(Received July 1998 - Accepted October 1998)

ABSTRACT

Three commonly used techniques, namely atomic absorption spectrophotometry (AAS-Ashing and AAS-Non Ashing) and titrimetry (potassium permanganate titration) have been evaluated in this study to determine the calcium content in six food samples whose calcium levels ranged from 0 to more than 250mg/100g sample dry matter (DM) basis. An attempt was made to evaluate these three techniques of analysis for all different levels on the basis of accuracy, precision, reproducibility of results, simplicity of operation, economy, speed, sensitivity, specificity, and safety. Results show that AAS-Ashing is the most reliable technique for calcium determination as it is most accurate and detects more calcium compared to the other two techniques. Moreover, independent of calcium levels, potassium permanganate titration proved to be the second most reliable method and determinations could be made more precisely, but it suffered from interference by other ions. AAS-Non Ashing proved to be the least accurate technique of analysis. The latter technique, however requires the shortest sample preparation procedure.

Keywords : AAS-Ashing, AAS-Non Ashing, potassium permanganate titration, calcium, food.

**For correspondence and reprints*

INTRODUCTION

Reliable data on the nutrient composition of foods are important in many areas of endeavour including health assessment, formulation of appropriate institutional and therapeutic diets, nutrition education, food and nutrition training, epidemiological research on relationships between diet and disease, plant breeding, nutrition labelling, food regulation and consumer protection as well as for a variety of applications in agriculture, trade, research, development and assistance (Scrimshaw, 1994). Food composition tables and databases are available in most countries, yet the data they contain are invariably criticised as being too inaccurate for many purposes (Sevenhuysen, 1994); one of the reasons is that different workers have used different techniques of analysis and that the samples so analysed had undergone different sample preparations leading to varying degrees of nutrient losses without ignoring the inherent variable factors that affect food composition, namely growing conditions, stage of ripeness or product formulation that are commonly not specified.

Analysis plays an important role in the assessment and maintenance of food quality and safety, both in industry and for enforcement authorities at the national and international levels (Kirk & Sawyer, 1991). Results obtained are compared with standard values set by various bodies, e.g. Standards Bureau, FAO. Method of analysis is an important factor that can affect the values obtained.

Up to now, few studies have been done to compare different techniques of analysis for determining mineral content in foods.

In this study, the calcium content in six vegetable samples with different calcium levels, namely (i) Processed Peas *(Pisum sativum)* (ii) Indian Kale *(Colocassia spp.)*, black stem type (iii) Red Kidney Bean *(Phaseolus vulgaris)* (iv) Watercress *(Nasturtium officinale)* (v) Soya Bean *(Glycine spp.)* and (vi) Amaranth (*Amaranthus spp*.) were determined by atomic absorption spectrophotometry (Ashing and Non Ashing) and potassium permanganate titration. The main objective was to investigate whether there are any differences between the three techniques of analysis for the different calcium levels on the basis of precision, accuracy, reproducibility, ease of determination, rapidity of execution and expertise required.

MATERIALS AND METHODS

The samples were selected on the basis of their calcium contents (Table 1).

Table 1. Samples selected and their calcium levels

Calcium range (mg/100g DM)	Samples	Calcium content (mg/100g DM)
0-50	Processed Peas	33[a]
50-100	Indian Kale	67[b]
100-150	Red Kidney Beans	100[a]
150-200	Watercress	170[a]
200-250	Soya Bean	240[a]
250 and above	Amaranth	351[b]

Source : [a]Holland *et al.* (1991); [b] FAO (1982)

Sample collection and preparation

The samples, especially the leafy vegetables, were purchased fresh mainly from the market place early in the morning on the day of analysis. Moreover, samples were purchased from a single source to reduce variations. Leafy vegetables were thoroughly washed with distilled water and were separated into the edible and non-edible portions. Edible portions were used in the study (Table 2). As for processed peas, the cans were cut open and the contents were transferred to a plastic strainer where the liquid was drained off. Soya beans and red kidney beans were just removed from the plastic packaging material and used directly. Samples were oven dried at 75°C until constant weights were obtained. They were then ground into a fine homogeneous powder and stored in tightly stoppered plastic bottles for further analysis.

Table 2. Description of the materials used for analysis

Vegetables	Edible portion	Non-edible portion
Processed Peas	100%	Nil
Indian Kale	Stem and leaves	Outer skin of stem
Red Kidney Bean	100%	Nil
Watercress	Stem and leaves	Tough stems > 4mm Ø
Soya Bean	100%	Nil
Amaranth	Stem and leaves	Tough stems + roots

Calcium determination

Analysis of each sample was carried out in ten replicates for all the three techniques of analysis, namely by
(i) Titration against standardised 0.2M potassium permanganate (ISO 6490/1)
(ii) Atomic absorption spectrophotometer - Ashing procedure (ISO 6490/2)
(iii) Atomic absorption spectrophotometer - Non Ashing procedure (James,1995).

A calibration curve for use in AAS was plotted by aspirating into the flame, samples of solutions containing known concentrations of calcium, measuring the absorption of each solution and then plotting a graph of absorption against concentration. Readings of absorbance were taken only after the instrument was set to zero with the use of a blank; in this case, lanthanum chloride solution was used. Dilution was done for the six samples prior to reading since in most cases the absorbance values were too high and would not fit into the range of the calcium standard solutions.

Statistical design and analysis

A completely randomised design (CRD) was used with treatments being the three techniques of analysis, viz. AAS-Ashing, AAS-Non Ashing and potassium permanganate titration. The design was considered to be appropriate as the experimental material used was quite homogeneous, being taken from a single source. Ten replicates for each technique ensured a precise estimate of the experimental error.

The results were analysed by one-way analysis of variance (ANOVA) to compare the relative accuracies in average calcium content determination between the three techniques for each of the six samples. Fisher's protected least significant difference (LSD) was then used for pairwise comparisons between the different means.

The standard error of the mean (SEM) calcium content by the different techniques was also determined as a measure of precision. Reproducibility between the techniques was tested using the usual F-test for equality of variances (James, 1995).

RESULTS AND DISCUSSION

Determination of calcium content

Table 3 compares the values obtained from the present study with other studies. 95% confidence intervals for the 'true' mean calcium content are also displayed. The interpretation of the intervals is that if repeated determinations are carried out by each technique, then for about 95% of these repetitions, the random interval will enclose the 'true' mean calcium content.

The results show that AAS-Ashing detects more calcium than the other two methods but in general have wider confidence intervals which are thus less informative.

From Table 3, it is observed that the values in the present study are fairly close to those of other workers for some of the samples, e.g. Soya Bean which has a mean calcium content of 237.7mg/100g DM basis with standard deviation 8.09 while Holland *et al*. (1991) obtained 240mg/100g DM basis. However, there is some degree of variation in the other samples. One has to be cautious when comparing values from different studies. Different plant parts may have been used e.g.for Indian Kale, FAO (1982) used the raw tuber and Ensminger *et al.* (1994) used the boiled drained leaves with stems while in this study, the leaves and the stems without the outer skin were used. Moreover some other factors that may influence the values are climatic conditions prevailing at time of harvest, soil conditions, nutrient status and degree of maturity of plant at harvest.

Another important factor that may account for the different values is that the workers did not specify the method of calcium determination nor the sample preparation, except Holland *et al*. (1991) who reported that calcium was determined by three techniques : AAS, titrimetry and ICPOES (Inductively Coupled Plasma Optical Emission Spectrophotometry) but did not specify the respective values for the different techniques.

Table 3. Comparison of the calcium content (mg/100g edible portion DM basis) of the samples in this study with other studies

	Processed Peas	Indian Kale	Red Kidney Beans	Watercress	Soya Bean	Amaranth
1a	19.2 (18.91,19.44)	69.1 (68.85, 69.32)	113.7 (113.47,114.01)	155.0 (154.74,155.27)	196.2 (196.00,196.46)	391.1 (390.67,391.51)
1b	13.3 (13.09,13.51)	53.8 (53.46,54.04)	99.4 (98.86,99.92)	116.1 (115.67,116.59)	170.0 (169.21,170.69)	352.6 (351.11,354.09)
1c	28.1 (27.56,28.66)	81.8 (80.49,83.05)	129.3 (125.08,133.48)	193.7 (190.08,197.34)	237.7 (231.92,243.50)	445.6 (441.95,449.19)
2	*	67	75	165	220	351
3	*	98	120	64	183	410
4	33	*	140	220	*	*
5	20	31	113	180	201	*
6	33	*	100	170	240	*
7	25	*	140	220	*	*
8	25	134	*	151	*	313

1 = Present study by:
a - potassium permanganate titration
b - AAS: Non Ashing
c - AAS: Ashing
2 = FAO (1982)
3 = Callikan (1982)
4 = MAFF (1985)
5 = Scherz & Senser (1994)
6 = Holland *et al.* (1991)
7 = Bender & Bender (1992)
8 = Ensminger *et al.* (1994)
* = Data unavailable
Figures in brackets are 95% confidence intervals for the means

Evaluation of the three techniques of calcium determination

During assessment of an analytical method, particular consideration often needs to be given to its precision, accuracy and reproducibility (James, 1995). Several statistical procedures are available for treatment of data to measure these parameters.

Precision

The term precision is defined as the closeness to each other of a number of replicate measurements, and is affected mainly by random errors associated with the analytical method (James, 1995).

It is desirable that this criterion be considered in choosing a particular analytical procedure as it is a measure of the ability to reproduce an answer between determinations performed by the same scientist or by different scientists in the same laboratory using the same procedure and instrument(s) (James, 1995).

From Table 4, in general, the means have relatively small standard errors indicating that the estimates of the actual calcium content in the different samples were determined precisely.

Table 4. Mean calcium content in samples with SEM

	Processed Peas[1]	Indian Kale	Red Kidney Beans	Watercress	Soya Bean	Amaranth
AAS- Ashing	28.1 ± 0.24a[2]	81.8 ± 0.57a	129.3 ± 1.86a	193.7 ± 1.61a	237.7 ± 2.56a	445.6 ± 1.60a
Titrimetry	19.2 ± 0.12b	69.1 ± 0.11b	113.7 ± 0.12b	155.0 ± 0.12b	196.2 ± 0.10b	391.1 ± 0.19b
AAS- Non Ashing	13.3 ± 0.10c	53.8 ± 0.13c	99.4 ± 0.24c	116.1 ± 0.21c	170.0 ± 0.33c	352.6 ± 0.66c

[1] Mean ± SEM based on n = 10 obs, expressed as mg /100 g DM basis; [2] Means shared by a common letter in each column are not significantly different at $P < 0.05$

AAS-Ashing seems to be less precise than AAS-Non Ashing because during ashing, temperature may cause volatilization of certain elements and may cause the mineral matter to melt and fuse. When fusion occurs in the presence of un-oxidised matter, the fused ash may surround and completely enclose or occlude some of the un-oxidised material. Such occluded matter is then protected from further oxidation by conditions normally used in ashing and the resulting ash will be too high. Ashing

temperatures can also influence the decomposition of inorganic compounds. Moreover precision could also be affected by the handling of the light fluffy ash which can be easily blown out of the dish during the weighing process.

It is also noted that lower precision is generally obtained with increasing calcium content in the case of AAS-Ashing and AAS-Non Ashing.
The SEM are relatively constant in the case of potassium permanganate titration indicating that the mean calcium content in the samples is determined with nearly the same precision, irrespective of its level. These results are also reflected in narrower 95% confidence intervals for the mean calcium content in this case (Table 3). The difference in precision using AAS and titrimetry may be due to the fact that dilution was carried out before reading the absorbance in the AAS.

Accuracy of the techniques of analysis

Another important criterion to consider is the accuracy of the techniques, that is expressed in terms of the ability to measure what is intended (James , 1995).
To compare the relative accuracies of the three techniques, a one-way ANOVA was carried out on data on the three techniques separately for each of the six samples. Significant differences ($P< 0.05$) were observed between the three techniques for each of the six samples.

Fisher's protected LSD for pairwise comparisons between means for the three techniques show that the mean calcium content for all six samples was significantly higher ($P< 0.05$) using AAS-Ashing (Table 4).

AAS-Ashing seems to be the most accurate technique of calcium determination followed by potassium permanganate titration. AAS-Non Ashing is the least accurate of all the three techniques.

Reproducibility of the techniques of analysis

Reproducibility may be defined as a comparison of the precision between two techniques. It may be estimated statistically by performing the F-test in order to compare the variances of the sets of data. The basic assumption or null hypothesis is that there is no significant difference between the variances of the two sets of data and, therefore, in the relative precision of the two techniques (James, 1995).

The F-test for equality of variances was conducted, at the 5% level of significance between the different techniques for all six samples (Table 5).

Table 5. Variances of different samples

Samples	AAS-Ashing*	AAS- Non Ashing	Titrimetry
Processed Peas	0.59	0.09	0.14
Indian Kale	3.21	0.16	0.11
Red Kidney Beans	34.50	0.55	0.14
Watercress	25.83	0.42	0.14
Soya Bean	65.39	1.09	0.11
Amaranth	25.57	4.38	0.35

*expressed as mg/100g DM basis

It was noted that AAS-Non Ashing and potassium permanganate titration gave significantly higher precision ($P< 0.05$) than AAS Ashing. This may be due to the fact that more preparation steps were involved in the Ashing procedure and thus the likelihood of errors by the analyst is higher. Moreover, the temperature changes, degree of volatilization and decomposition which commonly occur during the dry ashing procedure may vary and this may account for the lower reproducibility in AAS-Ashing. In the case of comparisons between potassium permanganate titration and AAS-Non Ashing, no significant differences in precision were found for Processed Peas and Indian Kale only, indicating that reproducibility for these two techniques are similar for very low and low calcium foods.

Simplicity of operation

Both AAS and potassium permanganate titrimetry require skilled analysts. Training is required for the manipulation of the atomic absorption spectrophotometer, especially regulation of the flow rates of fuel and oxidant as well as for the calibration of the equipment before sample reading. Less supervision is required in the case of AAS-Ashing compared to AAS-Non Ashing. Potassium permanganate titration requires a trained analyst in order to properly detect the end-point as well as for the preparation and standardisation of reagents.

Economy

AAS involves heavy investment costs in terms of the apparatus itself, proper laboratory facilities but also trained personnel and regular maintenance. Hence it is more expensive to carry out analysis by this method than by titrimetry. The latter method involves the use of numerous chemicals but the costs are not likely to exceed that of AAS. Moreover laboratory personnel need not be highly trained in order to carry out the analyses.

Speed

AAS-Non Ashing is the fastest technique of determination since it involves less sample preparation and skips the ashing step which is quite time-consuming. It took about 4h from sample preparation (after drying) to reading in the atomic absorption spectrophotometer for each batch of ten replicates. AAS-Ashing is a somewhat longer procedure since it involves the ashing procedure (a minimum of about 6h) while potassium permanganate titration is the longest and most time-consuming technique, especially the sample preparation procedure. Hence AAS can be said to be a faster method of analysis; approximately 150 samples can be read in one hour once the standard solutions have been read.

Sensitivity

AAS is more sensitive than potassium permanganate titration. The sensitivity of AAS using an air/acetylene flame is 8×10^{-8}g (Kenkel, 1992) while James (1995) quotes a value of 10^{-3}g as being that for titrimetric analysis. Therefore levels as low as 8×10^{-8}g will be detected by AAS while potassium permanganate titration will remain insensitive to such a level. Hence the use of AAS would be strongly recommended in cases where very low to low calcium levels are to be determined. However, AAS-Ashing is more sensitive to the nature of the sample compared to AAS-Non Ashing (Gorsuch, 1976).

Specificity

In the titrimetric technique, calcium has to be precipitated as calcium oxalate and then reacted with sulphuric acid so that the oxalic acid liberated is used for titration against permanganate ions. The reaction takes place in two steps and thus there are

several interference factors that affect the reaction, namely other ions having similar size and/or charge density as the calcium ions, e.g. magnesium, and especially sodium if present in greater concentration than calcium (Skoog & West, 1963). Phosphate ion interferes by competing with oxalate ions for binding with calcium ions. Moreover, the titration step has to take place at 70-80°C, for completion. Hence, if insufficiently heated, manganate compounds will be formed.

From Table 3, values obtained by AAS-Ashing are highest in all the six samples irrespective of calcium levels, implying that the technique detects more calcium than the other two. This may be due to the fact that spectrophotometry allows determination of calcium ions that acquire energy from the hollow cathode lamp and are then detected by the read-out device. Interferences are decreased to a very low level in AAS by using releasing agents, e.g. lanthanum chloride.

AAS-Non Ashing procedure gave the lowest values for calcium. Marked differences in values of calcium content were observed between AAS-Ashing and AAS-Non Ashing. In the latter procedure, where extraction is done by concentrated hydrochloric acid, problems may arise if insufficient calcium is extracted from the samples or if the reaction time is too short or even the volume of extracting liquid is insufficient. It is obvious that sample preparation can affect values since samples treated by both procedures were all read in the atomic absorption spectrophometer using the same calibration curve. The titrimetric technique seems to be better than AAS-Non Ashing since it detects more calcium.

Safety

Both AAS and permanganate titration require careful handling of inflammable gases and corrosive acids. Hence proper safety measures should be taken for both techniques.

Official approval

AAS-Non Ashing is not an official technique of analysis, but it is still being used. It was quoted by James (1995) and is not recognised by ISO, AOAC, or BSI, unlike AAS-Ashing and potassium permanganate titration which are both established ISO procedures. The use of an official method or technique ensures that uniform procedures are used during an experiment and provides a basis for further investigations and discussion of the results.

CONCLUSION

The eventual choice of a method or technique depends on several factors and on the purpose of the experiment. If required for official reports, matters of dispute or involving legislative requirements, the use of an officially approved method or technique can be of utmost importance while for routine analysis in quality control, speed, cost and precision could have a more important bearing. The analyst would most probably choose AAS-Ashing for calcium determination for any level out of the three techniques investigated if characteristics such as accuracy, speed, sensitivity, specificity and official approval are required. In the absence of the 'true' calcium content AAS-Ashing seems to be the best technique as it could detect higher calcium values irrespective of the calcium levels and its values are closest to those quoted in literature (Table 3). However, AAS-Ashing seems to suffer from a lower precision than potassium permanganate titration since there was much variability in the results obtained.

Potassium permanganate titration is a also reliable technique and does not involve heavy investment costs but the colour at attainment of end-point is a subjective factor and may become a matter of dispute. Frequent standardisations are required prior to use. Moreover sample preparation is lengthier when compared to AAS and requires precipitation of the element to be analysed. It has been used for many years before the invention of the atomic absorption spectrophotometer and is still being used in laboratories which cannot invest in such equipment like the atomic absorption spectrophotometer or where use of the latter will not be regular.

AAS-Non Ashing gave the lowest results for calcium content and was the least accurate technique. However it is similar in reproducibility to potassium permanganate titration for Processed Peas and Indian Kale, i.e., very low and low calcium foods respectively. This technique requires the shortest sample preparation and can be used only where fast approximate values are required since it skips the ashing step which is quite time-consuming. Hence the use of AAS-Non Ashing can be recommended in cases where foods have to be screened for their calcium levels.

This study has confirmed that atomic absorption spectrophotometry (Ashing technique) is a very good method of calcium determination if interference factors are eliminated with the help of releasing agents. Nevertheless potassium permanganate titration is a reliable method too and its use can be recommended where AAS is not available. Very often research workers gives analytical values without specifying the method used. This study confirms that different analytical techniques can detect different amounts of calcium in food. Sample preparation as

clearly shown in AAS-Ashing and Non Ashing also influences the amount of calcium detected in food. Thus it is imperative that methods of analysis should be clearly specified whenever analytical values are presented.

REFERENCES

BENDER, A.E. & BENDER, D.A. (1992). *Food Tables.* Oxford University Press, UK.

CALLIKAN, S. (1982). Representative composition of fruits and vegetables commonly consumed in Mauritius. *Organe Officiel de la Société de Technologie Agricole et Sucrière de l'Ile Maurice* **61** (2), 71-78.

ENSMINGER, A.H., ENSMINGER, M.E., KONLANDE, J.E. & ROBSON, J.K. (1994). *Foods and Nutrition Encyclopaedia.* CRC Press, USA.

FAO (1982). Food Composition Tables for Near East. *Food and Nutrition* **26**.

GORSUCH, T.T. (1976). *Accuracy in Trace Analysis Sampling, Sample Handling and Analysis*. National Bureau of Standards, Washington.

HOLLAND, B., PAUL, A.A., SOUTHGATE, D.A., BUSS, D.H. & UNWIN, I.D.(1991). *Mc Cance and Widdowson's The Composition of Foods.* Royal Society of Chemistry & MAFF, UK.

JAMES, C.S. (1995). *Analytical Chemistry of Foods.* Chapman & Hall, USA.

KENKEL, J. (1992). Atomic Spectroscopy. In *Analytical Chemistry Refresher Manual,* p 187. Lewis Publishers, USA.

KIRK, R.S. & SAWYER, R. (1991). *Pearson's Composition and Analysis of Foods*. Longman Scientific and Technical, UK.

MINISTRY OF AGRICULTURE, FISHERIES AND FOODS (MAFF) (1985). *Manual of Nutrition*. Her Majesty's Stationery Office, UK.

SCHERZ, H. & SENSER, F. (1994). *Food Composition and Nutrition Tables.* Munchen: Deutsche Forschungsanstalt für Lebensmittelchemie.

SCRIMSHAW, N.S. (1994). The importance of the International Network of Food

Data Systems. In *Food, Nutrition and Agriculture - Food Composition Data,* p 6. *(*Ed J.L. ALBERT). FAO, Rome.

SEVENHUYSEN, G.P. (1994). Food composition databases: current problems and solutions. In *Food, Nutrition and Agriculture - Food Composition Data,* p 21. (Ed J.L. ALBERT). FAO, Rome.

SKOOG, D.A. & WEST, D.M. (1963). *Fundamentals of Analytical Chemistry*. Holt, Rinehart and Winston Inc, USA.