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Determination of Calcium in Foods by the Atomic Absorption Spectrophotometric and Titrimetric Methods

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ABSTRAK

Laporan ini membentangkan hasil satu kajian perbandingan penentuan kandungan kalsium dalam pelbagai jenis makanan dengan kaedah-kaedah spektrofotometri penyerapan atom (AAS) dan titratan dengan kalium permanganat. Larutan abu telah disediakan bagi setiap sampel makanan (dianalisis secara duplikat). Satu alikuot larutan tersebut telah dianalisis dengan kaedah AAS, manakala satu lagi dengan kaedah titratan. Sejumlah 132 jenis makanan yang terdiri daripada 8 kumpulan makanan telah dikaji. Nilai min bagi analisis duplikat setiap makanan dengan kedua-dua kaedah itu telah dibentangkan mengikut kumpulan makanan. Hasil yang diperolehi dengan kaedah AAS dan titrimetri didapati mempunyai keselarian dan korelasi yang baik ($r = 0.998$). Ini telah disahkan dengan ujian "paired t" yang menunjukkan bahawa bagi 6 kumpulan makanan yang dikaji, perbezaan kandungan kalsium yang diberi oleh kedua-dua kaedah tidak bererti ($p < 0.05$). Walaupun begitu perbezaan bagi 2 kumpulan yang lain iaitu kekacang dan sayuran didapati bererti dan angka statistik t yang diperolehi kecil. Kedua-dua kaedah didapati memberi perbezaan min hasil bilas yang tidak bererti dan menghampiri 100. Didapati juga perbezaan yang tidak bererti bagi varians kaedah-kaedah itu. Hasil kajian ini telah menunjukkan bahawa kedua-dua kaedah dapat digunakan dengan memuaskan bagi analisis zat ini. Walaupun demikian, pilihan sesuatu kaedah juga bergantung kepada beberapa faktor yang lain, termasuk adanya alat dan kepakaran yang diperlukan.

ABSTRACT

This report presents results of a comparative study of the determination of calcium in a wide variety of foods using the atomic absorption spectrophotometric (AAS) and potassium permanganate titration methods. Ash solution for each food sample (determined in duplicate) was prepared and an aliquot subjected to AAS analysis, while another aliquot was determined by the titrimetric method. A total of 132 foods, belonging to 8 food groups were studied. Mean values for duplicate analysis of each food determined by the two methods were tabulated according to food groups. Results obtained by the AAS and titrimetric methods showed good general agreement, and a high correlation coefficient ($r = 0.998$) was obtained. This was confirmed by paired t-test which showed that for 6 of the food groups studied, there was no statistically significant difference ($p < 0.05$) in calcium concentrations determined by the two methods. For the remaining 2 groups, legumes and vegetables, a significant difference in results was obtained. However, in both cases, the t-statistic calculated was small. Both methods were found to give mean percent recovery values which were not significantly different and close to 100. There was also no significant difference in variances given by the two methods. Results of the study therefore have shown that either method can be used satisfactorily for the analysis of this nutrient. The choice of method, however, also depends on various other factors, including availability of required instrument and expertise.

INTRODUCTION

Calcium has been documented in studies of nutrient composition of local foods since the early part of the century. One of the earliest reports was that of Morris and Oliveiro (1933) who documented the content of this mineral in

some 60 types of foods. In that study, calcium was precipitated as calcium oxalate, converted to calcium oxide, weighed and reported as such. Some years later, Leong and Morris (1947) used a different procedure for determining this mineral. Calcium was again precipitated as oxalates, but instead of using the more cumbersome gravimetric procedure, calcium present was next titrated with potassium permanganate and results expressed as milligram calcium. Subsequent reports on nutrient analyses of local foods had used this titrimetric method for determining calcium.

The potassium permanganate titration method (after precipitation of calcium as oxalate) has remained the method of choice for determination of calcium in foods for many laboratories, including this Division. In recent years, the atomic absorption spectrophotometric (AAS) method has been introduced. This, and the titrimetric methods, are recognized methods for determination of calcium in foods, and are cited in Pearson's Chemical Analysis of Foods (Egan *et al.* 1981). Both methods are currently in use by laboratories in the country carrying out studies into nutrient composition of foods.

The choice of either the AAS or titrimetric method has relied on various factors, including availability of the required instrument as well as expertise. For various reasons, it would be important to determine if the AAS and titrimetric methods give comparable results. Different laboratories participating in a joint programme for the analysis of calcium using the two different methods would need to determine if the results obtained are comparable. Before switching over to a newly purchased atomic absorption spectrophotometer, a laboratory would need to find out if the results to be obtained would be comparable to those previously obtained with the titrimetric method. On the other hand, in a laboratory using the AAS method, it may be necessary to switch to the titrimetric method if the spectrophotometer breaks down for a considerable length of time.

This report presents results of a comparative study of the determination of calcium in a wide variety of foods using the AAS and titrimetric methods. It is hoped that the results indicate clearly significant differences, if any, between the two analytical methods. This could be of assistance to laboratory workers intending to use either methods, such as in situations mentioned above. The study was carried out together with a comparative study of the determination of iron using the AAS and colorimetric methods (Tee *et al.* 1989).

MATERIALS AND METHODS

Samples of foods from various food groups were purchased from local markets and retail stores for analysis. Wherever applicable, refuse in each food item was removed and its proportion in the food determined. The edible portions were blended and aliquots taken for analysis.

An amount of 5-15 g of the homogenized sample was dried in an air oven at 105°c for 3 hours. The dried sample was next charred until it ceased to smoke. The charred sample was then ashed in a muffle furnace at 550°C until a whitish or greyish ash was obtained. The ash was treated with concentrated hydrochloric acid, transfered to a volumetric flask and made up to 50 ml. For each food studied, two ash solutions were prepared, i.e. duplicate analysis was carried out. An aliquot of each ash solution was used for the determination of calcium by the AAS method and another aliquot by the titrimetric method.

For the AAS method, a Varian Atomic Absorption Spectrophotometer model 175 with an air-acetylene flame, and wavelength set to 422.7 nm was used. Calcium carbonate was used as standard to prepare a calibration curve with at least 4 concentrations of calcium within the analytical range. To eliminate phosphorus interference in the determination, lanthanum was added to the test ash solution and standard solutions so that the final solutions contained 1% La. Concentration of calcium in test solutions was calculated from the standard curve prepared. For each ash solution, at least three readings were obtained and the average cal culated.

In the titrimetric method, an aliquot of the ash solution was reacted with ammonium oxalate solution to precipitate out the calcium. After centrifugation and decanting the supernatant liquid, the precipitate was redissolved in 4N sulphuric acid. Calcium in solution was titrated against 0.01N potassium permanganate,

with the solution kept at about 75-85°C throughout the titration. For each ash solution prepared, at least two titrations were carried out to determine the average titre. Standard solutions of calcium carbonate were similarly titrated and the titre used for calculation of calcium in the test ash solutions.

Recovery studies were performed by adding a known amount (about 50% of the estimated calcium content of the food) of calcium stock standard to the food. Preparation of ash solution and analysis of calcium using the AAS and titrimetric methods were carried out as described above.

Details of the AAS and potassium permanganate titration methods used are described in the laboratory manual in use in this Division (Tee et *al.* 1987). All results were expressed as per 100 g edible portion of the food. Mean values for duplicate analysis of each food determined by the two methods were calculated and results tabulated according to food groups. For each food group, the paired t-test was carried out using the ABSTAT statistical programme to determine if the two methods gave significantly different results. Correlation coefficient was calculated using the same programme. Analytical process standard deviations of the two methods were compared using the F-ratios method (Wernimont 1985).

RESULTS AND DISCUSSION

A wide variety of foods from various food groups were studied, to determine if different food matrixes would affect the results obtained. A total of 132 foods, belonging to 8 food groups were studied. Mean values for duplicate analysis of each food determined by the AAS and titrimetric methods were tabulated according to food groups (Tables 1 to 8). In all the tables, the English names of the foods are given, and arranged in alphabetical order. Where these names may be ambigious or unclear, or when the English names are not known, the local names of the foods have been included. The scientific names of the foods are also tabulated where appropriate.

There was generally good agreement in the results obtained by the two methods (Tables 1 to 8). This is clearly seen in the scatter dia-

TABLE 1
Calcium in cereals and products as determined by the atomic absorption spectrophotometri and titrimetric methods

English/local name	mg Ca/100 g edible portion	
	AAS method	Titrimetric method
Bread, coconut	17.7	15.9
Bread, ryemeal	49.0	50.3
Bread, white	43.9	42.3
Bread, wholemeal	38.2	38.9
Noodle *laksa*, thick, dry	10.3	9.2
Noodle *laksa*, thick, wet	5.1	4.0
Oats, processed, tinned	49.6	47.6
Oats, rolled	39.7	38.7
Rice, broken	7.5	7.1
Rice bran, coarse	50.4	51.3
Rice bran, fine	45.2	51.1
Rice noodle (*Loh-see-fun*)	4.6	3.7
Wheat flour, high protein	26.9	24.8
Wheat flour, wholemeal	45.5	42.9
Wheat germ	55.2	53.3

Each value is the mean of duplicate analysis

TABLE 2
Calcium in legumes and products as determined by the atomic absorption spectrophotometric and titrimetric methods

	mg ca/100 g edible portion	
	AAS method	Titrimetric method
Baked beans, canned	42.4	40.5
Chickpea/Common gram	132.9	127.6
Dhal, Mysore	30.6	24.8
Soya bean, fermente (Tempeh)	75.1	70.8
Soya bean cake (*Tau-Kua*), spiced	179.9	160.8
Soya bean cake (*Tau-kua*)	183.7	156.9
Soya bean curd sheets (*Fucok*)	224.7	191.3
Soya bean curd, strands (*Fucok*)	262.7	239.3
Soya bean curd (*Tau-hoo-fa*)	67.3	66.3
Soya bean curd (*Tau-hoo-pok*)	82.1	77.0
Soya bean milk, packet	6.3	5.1
Soya bean milk, unsweetened	14.4	12.5
Soya bean noodles	25.0	25.7

Each value is the mean of duplicate analysis

TABLE 3
Calcium in nuts and seeds as determined by the atomic absorption spectrophptometric and titrimetric methods

English/local name	Scientific name	mg Ca/100 g edible portion	
		AAS method	Titrimetric method
Almond	*Prunus amygdalus*	243.1	221.9
Arecanut shavings	*Areca catechu*	39.6	43.6
Brazil nut	*Bertholletia excelsa*	173.9	200.9
Candlenut	*Aleurites moluccana*	152.6	148.5
Cashew nut	*Anacardium occidentale*	37.0	35.1
Chestnut, Chinese	*Castanea spp.*	15.8	16.0
Coconut cream	*Cocos nucifera*	6.6	6.8
Coconut flesh, old	*Cocos nucifera*	9.1	9.4
Coconut flesh, young	*Cocos nucifera*	20.5	19.5
Coconut milk	*Cocos nucifera*	8.0	8.8
Coconut water	*Cocos nucifera*	15.2	13.2
Lotus seed	*Nelumbo nucifera*	131.6	117.4
Peanut butter	*Arachis hypogea*	45.7	45.4
Sesame seed/Gingelly seed	*Sesamum indicum*	55.3	53.0
Walnut, dried	*Juglans regia*	131.9	118.5
Watermelon seed, black, dried	*Citrullus vulgaris*	56.7	53.8

Each value is the mean of duplicate analysis

TABLE 4
Calcium in vegetables as determined by the atomic absorption spectrophotometric and titrimetric methods

English/local name	Scientific name	mg Ca/100 g edible portion	
		AAS method	Titrimetric method
Asparagus, canned	*Asparagus officinalis*	14.7	13.7
Asparagus, fresh	*Asparagus officinalis*	13.9	12.3
Drumstick, fresh pods	*Moringa oleifera*	23.8	22.4
Gourd, bottle/Calabash	*Lagenaria vulgaris*	15.8	14.42
Kadok, leaves	*Piper sarmentosum*	246.1	219.5
Leek	*Allium porrum*	16.2	15.8
Mushrooms, grey oyster, fresh	–	1.0	2.2
Peas, garden, fresh	*Pisum sativum*	62.5	58.7
Purslane	*Portulaca oleracea*	76.1	72.4
Radish, Chinese, pickled	*Raphanus sativus*	94.9	98.8
Rhubarb/Pie plant, petioles	*Rheum rhaponticum*	268.9	253.0
Seaweed, agar (Agar-agar)	–	510.2	502.1
Spinach, Ceylon	*Basella rubra*	116.2	112.5
Spinach (*Bayam pasir*)	–	318.4	287.2
Tree tomato	*Cyphomandra betacea*	11.2	11.6
Yam bean	*Pachyrrhizus erosus*	12.4	12.5

Each value is the mean of duplicate analysis

TABLE 5
Calcium in fruits as determined by the atomic absorption spectrophotometric and titrimetric methods

English/local name	Scientific name	mg Ca/100 g edible portion	
		AAS method	Titrimetric method
Avocado	*Persea americana*	13.8	12.1
Banana (*Pisang kelat*)	*Musa sapientium*	5.7	6.8
Binjai	*Mangifera caesia*	6.9	6.7
Cashew apple	*Anacardium occidentale*	2.0	2.0
Custard apple	*Annona squamosa*	16.4	15.5
Date, dried	*Phoenix dactylifera*	47.6	42.2
Durian cake	*Durio zibethinus*	9.4	11.1
Grapefruit	*Citrus paradisi*	28.5	26.8
Jering	*Pithecellobium lobatum*	31.3	38.1
Kundang	*Bouea macrophylla*	4.9	5.2
Lychee	*Litchi chinensis*	5.1	5.1
Mango (*Bacang gelok*)	*Mangifera foetida*	16.0	15.5
Nutmeg, fresh	*Myristica fragrans*	26.8	24.1
Persimmon, dried	*Diospyros kaki*	43.1	36.1
Prunes, dried	*Prunus spp.*	56.3	52.4
Pulasan	*Nephelium mutabile*	7.8	7.1
Soursop	*Annona muricata*	12.0	10.6
Strawberry	*Fragaria grandiflora*	12.0	11.9

TABLE 6
Calcium in meat and eggs as determined by the atomic absorption spectrophotometric and titrimetric methods

	mg Ca/100 g edible portion	
	AAS method	Titrimetric method
Beef extract	40.4	43.2
Beef rendang, canned	31.1	26.6
Chicken feet, deboned	25.1	23.3
Chicken gizzard	7.4	7.2
Chicken heart	6.0	6.0
Chicken intestines	7.7	5.7
Duck egg, salted, yolk	184.1	188.7
Duck egg, yolk	151.3	139.7
Mutton curry, canned	16.1	15.6
Ox maw	10.7	9.7
Turtle egg, white	19.6	21.0
Turtle egg, yolk	165.2	157.9

Each value is the mean of duplicate analysis

TABLE 7
Calcium in fish and fish products as determined by the atomic absorption spectrpohotometric and titrimetric methods

English/local name	Scientific name	mg Ca/100 g edible portion	
		AAS method	Titrimetric method
Anchovy, cleaned, dried	*Stolephorus commersonii*	547.5	500.5
Anchovy, whole, dried	*Stolephorus commersonii*	1238.1	1255.2
Cuttlefish, dried	*Sepia officinalis*	103.1	95.7
Fish balls	–	58.5	56.5
Fish bladder, dried	–	19.3	19.0
Fish bladder, fried	–	18.0	20.6
Fish curry, canned	–	338.0	320.8
Fish roe	–	13.1	12.6
Fish sauce (*Budu*)	–	390.6	383.9
Hairtail scad, dried	*Megalaspis cordyla*	95.6	98.1
Live crab/Swimming crab	–	232.8	226.3
Oyster sauce	*Ostrea spp.*	24.8	16.9
Oyster	*Ostrea spp.*	180.9	174.5
Prawn paste (*Hay-ko*)	–	286.1	325.9
Sea crab/Blue crab	–	168.7	167.6
Shark's fin, dried	–	418.1	425.5
Shrimp, fermented (*Cincalok*)	–	450.3	475.3
Threadfin, dried	*Polynemus indicus*	29.3	34.8
Yellow banded trevally, dried	*Selaroides leptolepis*	157.4	150.4

Each value is the mean of duplicate analysis

TABLE 8
Calcium in miscellaneous foods as determined by the atomic absorption spectr photometric and titrimetric methods

English/local name	Scientific name	mg Ca/100 g edible portion	
		AAS method	Titrimetric method
Anise seed, dried	*Pimpinella anisum*	950.6	1004.8
Cardamon	*Elettaria cardamomum*	1769.7	1704.0
Choocolate, raisin	–	182.6	178.4
Cinnamon	*Cinnamomum zeylanicum*	600.9	534.0
Coffee mixture, powder	–	167.5	180.2
Cumin seeds, black	*Nigella sativa*	816.8	818.1
Cumin seeds, white	*Cuminum cyminum*	1165.1	1093.3
Curry powder	–	576.2	560.1
Fenugreek seeds	*Trigonella foenum-graecum*	179.8	174.8
Galangal	*Languas galanga*	12.8	9.5
Honey	–	7.0	8.6

Continued on next page

TABLE 8: Continued

English/local name	Scientific name	mg Ca/100 g edible portion	
		AAS method	Titrimetric method
Jam, egg (*Seri kaya*)	–	8.4	8.0
Jam, pineapple	–	1.7	3.3
Jelly crystals	–	133.2	124.0
Malted milk powder	–	501.9	488.1
Marmalade	–	7.9	7.7
Milk-based diet supplement, powder	–	761.3	711.1
Pepper, powder, white	*Piper nigrum*	120.4	122.1
Sugar cane juice	*Saccharum officinarum*	6.5	6.1
Tamarind paste (*Asam Jawa*)	*Tamarindus indica*	101.7	89.2
Treacle, black	–	517.4	487.6
Yeast, dried, brewer's	*Saccharomyces cerevisiae*	400.7	420.2
Yeast, granules, tinned	*Saccharomyces cerevisiae*	68.6	65.5

Each value is the mean of duplicate analysis

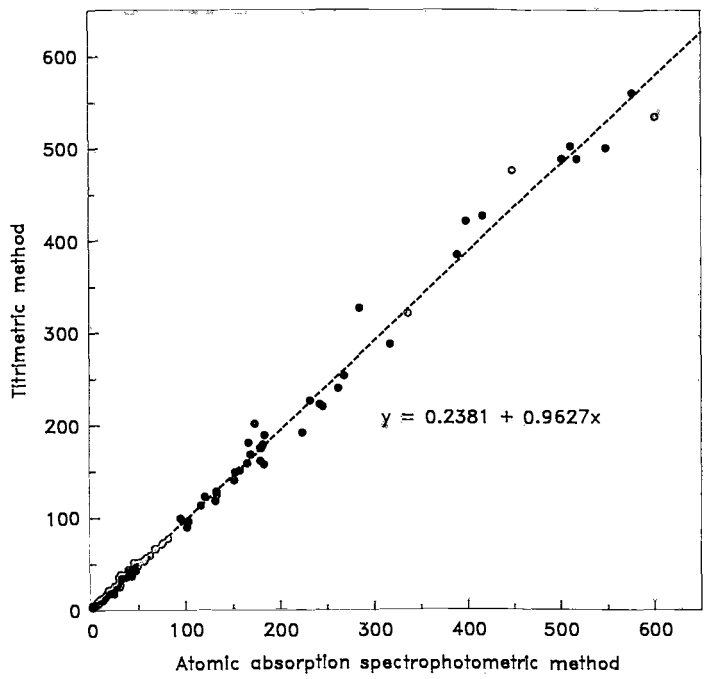

Fig. 1: Calcium concentration determined by the AAS and Titrimetric methods (mg Calcium per 100 g edible portion).

gram, plotting 126 pairs of results obtained (*Figure 1*). The remaining 6 pairs were omitted from the plot as they were much higher than the majority of the values obtained. A good correlation coefficient ($r = 0.998$) was obtained for all 132 pairs of results obtained.

Results of paired t-test for all food groups studied (Table 9) showed that for 6 food groups, there was no statistically significant difference ($p < 0.05$) in calcium concentration determined by the AAS and titrimetric methods. For the remaining 2 groups, legumes and vegetables, a significant difference in results was obtained. However, in both cases, particularly for vegetables, the t-statistic calculated was small, just above the significance level.

Recovery of added calcium to the foods was determined in 14 separate studies. Results obtained (Table 10) showed that mean percent recovery values for both methods were close to 100, with small coefficient of variation. There was no statistically significant difference between the two mean recovery values ($p < 0.05$).

The pooled standard deviation obtained for all the 132 foods studied was 14.5 for the AAS method and 15.8 for the titrimetric method. Comparing the variance obtained for all foods, the observed F-ratio was calculated to be 1.17. There was thus no statistically significant difference ($p < 0.05$) in the variances given by the two methods.

CONCLUSIONS

In this study, the AAS and the potassium permanganate titration methods did not give significantly different calcium concentrations for a wide variety of foods. Both methods gave good recovery values, and no significant difference in process variablility was observed. Either method can, therefore, be used satisfactorily for this analysis. There are, however, advantages and disadvantages for both methods.

The titration method tends to be more tedious and more prone to errors due to the number of steps involved in preparing the solution for titration. This include adjustment

TABLE 9
Summary statistics of paired t-test of calcium concentration of various foods determined by the atomic absorption spectrophotometric and titrimetric methods

Food group	n	Calculated t-statistic	Statistical significance[1]
Cereals and products	15	0.939	N.S[2]
Legumes and products	13	3.094	S[3]
Nuts and seeds	16	0.749	N.S.
Vegetables	16	2.312	S.
Fruits	18	1.312	N.S.
Meat and eggs	12	1.316	N.S.
Fish and fish products	19	0.134	N.S.
Miscellaneous	23	1.862	N.S.

[1] at $p < 0.05$
[2] not statistically significant
[3] statistically significant

TABLE 10
Recovery values obtained by the atomic absorption spectrophotometric and colorimetric methods

	AAS method	Titrimetric method
Number of determinations	14	14
Mean ± SD	96.9 ± 9.3%	93.5 ± 6.8%
Coefficient of variation	9.6	7.3

of pH of ash solution, precipitation of calcium as oxalate, and collection and cleaning of the precipitate. The titration itself has to be carefully performed, keeping the test solution at a temperature of 75-85°C. The procedure is, however, relatively much cheaper, requiring no expensive instrument. In the hands of an experienced worker, this method can provide reliable results.

The AAS method, on the other hand, requires the purchase of an expensive spectrophotometer. It has also to be borne in mind that maintaining the instrument to ensure optimal performance is a difficult task. It is however, a relatively simpler procedure. The ash solution can be used directly for spraying in the spectrophotometer, after the instrument has been appropriately set up. It would be the method of choice, provided the required budget is available.

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