ACCURATE DETERMINATION OF CALCIUM AND OTHER ELEMENTS IN CEREAL GRAINS

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ABSTRACT

The usual interference of phosphorus in the absorption of calcium in the flame was overcome by the addition of at least 2000ppm strontium to the sample solution giving results which are higher for calcium than those earlier reported in the literature for similar samples. Attention is drawn to the dangers posed by such published results and reliance on them for any purpose.

INTRODUCTION

Cereals are the most widely cultivated and consumed crops the world over and provide mainly energy and protein in diets. Okoh[1] projected that cereals were likely to continue to play increasing roles in developing countries of the tropics. Cereal grains contain less of the essential amino acids, particularly lysine, needed for growth and maintenance[2,3]. Maize, sorghum and millet are inferior to other grains because of their high prolamine content[1]. Cereals are known to contain most of the elements of nutritional interest. The major elements potassium, magnesium and calcium are present as phosphates and sulphates[1].

It was observed that the concentration of calcium in cereal grains reported in the literature[4-7] was suspiciously low compared to the quantity of ash produced by these substances. In cereals, most of the phosphorus is present as phytic acid[1] which also inhibits calcium absorption. It is common knowledge that phosphorus interferes with flame atomic absorption of calcium due to the formation of a refractory $Ca_2P_2O_7$. It is also common knowledge that the interference due to phosphorus is overcome by the addition of lanthanum or strontium to the matrix before analysis by atomic absorption in the air-acetylene flame. Such interferences have been overcome for a wide range of matrices including unused lubricating oils[8], vegetables[9], animal protein sources[10] and powdered milk[11]. Seeking to probe the suspicion of low calcium results mentioned earlier, a series of experiments were arranged to examine whether phosphorus has an effect on the [illegible] and be obtained. The purpose of this paper is to present a sample preparation procedure incorporating the use of strontium for the determination of calcium and other elements in cereal grains by atomic absorption and caution about the use of data available in the literature before now.

EXPERIMENTAL

Apparatus

A Jenway PF7 flame photometer (Jenway, England) was used for the determination of sodium and potassium. A Unicam 919 atomic absorption spectrophotometer (Pye Unicam, England) was used for the determination of all other test elements. Phosphorus was determined with a Unicam uv/v spectrophotometer equipped with a computer.

Reagents

All reagents were of analytical grade. Deionised water was used in all preparations and dilutions. 50000ppm strontium solution was prepared by dissolving 152.1[illegible]g $SrCl_2.6H_2O$ in water and diluting to 1liter in a volumetric flask.

Working solutions and calibration processes

1000ppm stock standard solutions of the elements were prepared as documented in the user's manual for the AAS used. Working standard solutions were prepared by serial dilution of the stock solutions. Calibration curves were prepared fresh for the subsequent analysis. Element responses from the samples were subsequently subjected to mathematical treatment to convert [illegible] concentrations.

Cereal samples

Cereal grains were purchased from the market, dried to constant weight in the oven and blended into fine powder. A full list is given in Table 1.

Determination of phosphorus

One gramme of each sample powder was weighed out in triplicate into crucibles. The samples were charred and eventually ashed in a muffle furnace at 550°C for 3h. Each ash was treated with 20.0ml 6M HCl and the solution concentrated on a steam bath to about 5.0ml. The solution was then transferred quantitatively into a 50ml volumetric flask and diluted to the mark with water. The resulting solutions were used for the determination of phosphorus by the yellow method of Kitson and Mellon[12]. The results are shown in Table 1 as averages of the triplicates.

Table 1. Phosphorus content of cereal samples.

Sample number	Sample name	Phosphorus content (ppm) x 10^2
1.	Sorghum bicolor (guinea corn)	49.80
2	Setaria italica (millet)	36.95
3	Fagopyrum sagittatium (wheat)	36.50
4	Zea mays (maize)	28.96
5	Oryza sativa (rice)	17.52

Effect of increasing concentration of strontium on the concentration of calcium and magnesium

One gramme of guinea corn (sample 1) was weighed out into ten crucibles and ashed at 550°C for 3h. Each ash was treated with 20.0ml 6M HCl and the solution concentrated on a steam bath to about 5.0ml. Varying concentrations of strontium were added and the resulting solution made up to 50ml with water. A blank solution was prepared and similarly treated. The solutions were used for the determination of calcium and magnesium. Average results of duplicate determinations are shown in Table 2.

Recovery studies

Known amounts of calcium (0.175g $CaCO_3$), magnesium (0.133g MgO), zinc (0.050g ZnO) and sodium (0.127g NaCl) calculated to contain 70, 80, 40 and 50mg of calcium, magnesium, zinc and sodium respectively, were added to 1.0g of sample 2 weighed out in triplicate and ashed. The resulting ash was dissolved and analyzed as detailed in the recommended procedure.

Table 2. Effect of strontium on the release of calcium and magnesium for analysis

Concentration of strontium, ppm	Concentration (ppm) x 10^2	
	Magnesium	Calcium
0	30.58	19.09
500	33.33	25.23
1000	33.10	31.14
1500	33.32	43.86
2000	32.99	68.68
2500	33.32	68.10
3000	34.08	69.32
4000	33.30	67.72
5000	43.09	69.78
6000	33.31	68.64

Reproducibility studies

One gramme of sample 3 was weighed out in six replicates, ashed and analysed for all elements of interest as described in the recommended procedure.

Application of procedure and comparison of results

One gramme of each sample in Table 3 was weighed out in duplicate, ashed and dissolved as described in the recommended procedure. A blank solution was also prepared and all the solutions were analyzed for the elements of interest by AAS.

Table 3. Sample results by the recommended procedure.

Sample number	Ca	Mg	Fe	Na	K	Zn	Mn	Cu
	Concentration (ppm) x 10^2							
1	68.71	33.43	16.77	248.50	139.59	8.80	2.15	0.80
2	42.00	30.25	14.16	198.50	126.18	7.84	2.79	0.81
3	52.00	37.50	11.96	148.50	89.59	8.08	2.94	1.17
4	42.00	24.00	8.18	298.50	101.61	8.44	2.34	0.92
5	32.00	30.25	10.06	198.50	44.13	7.36	2.62	1.01

Recommended procedure

One gramme of each sample is weighed into a crucible and put in a muffle furnace set at a temperature of 200°C to char and, then at 550°C until the ash is free from all visible traces of carbon. The crucible is removed from the furnace and allowed to cool. The ash is treated with 20.0ml 6M HCl and the resulting solution concentrated on a steam bath to about 5.0ml. The solution is transferred quantitatively to a 50ml volumetric flask, 2.0ml 50000ppm strontium solution is added and the resulting solution diluted to the mark with water. The resulting solution is analyzed for the elements of interest using atomic absorption. A blank solution is also prepared and analyzed.

RESULTS AND DISCUSSION

Determination of phosphorus

Table 1 shows the phosphorus content of the study samples. All the samples had relatively high phosphorus content with guinea corn having the highest and rice, the lowest. Experience has shown that the sample with the highest phosphorus content is likely to encounter the highest degree of interference by phosphorus in the determination of calcium by atomic absorption operated on the air-acetylene flame. Guinea corn was therefore used as pilot in further work. The phosphorus results compare closely (Table 4) with those for the same generic samples in the literature.

Table 4. Phosphorus, calcium and magnesium contents of study samples in comparison with earlier results in literature[1].

Sample name	Element/concentration, mg/100g dry mass					
	P		Ca		Mg	
	this work	others	this work	others	this work	others
maize	290.00	-	420.00	2.20[5]	240.00	377.00[5]
sorghum	498.00	-	687.00	8.20[5]	342.00	322.00[5]
millet	370.00	358.00[4]	420.00	50.00[4]	303.00	180.00[4]
rice	175.00	390.50[7]	320.00	51.00[7]	303.00	60.00[7]
wheat	365.00	392.50[6]	520.00	36.00[6]	375.00	170.00[6]

Effect of increasing concentration of strontium on the concentration of calcium and magnesium

The amounts of calcium obtained increased with increase in the concentration of strontium up to a peak value which remained appreciably constant with and beyond a strontium concentration of 2000ppm (Table 2). The effect of strontium on magnesium absorption was not significant and the table also shows that with or without the addition of strontium, the concentration of magnesium obtained was about the same. Magnesium is not listed as being affected by phosphorus interference[13]. Similar results have been reported for various other matrices[7-10]. Sample solutions containing 2000ppm strontium were used in further work.

Recovery studies

Average recoveries (%) were 100.00±0.00 for sodium and calcium, 99.24±2.76 for magnesium and 99.45±1.55 for zinc. Application of the student t-test showed that both added and recovered values were not significantly different at the 95% confidence level.

Reproducibility studies

The coefficients of variation calculated for the different elements were 0.00% for calcium, magnesium, sodium and potassium, 1.77% for copper, 2.47% for zinc, 4.81% for iron and 6.51% for manganese at the concentration levels found in that sample. These showed that the procedure gives highly reproducible results.

Application of procedure and comparison of results

The results in Table 3 show wide variations in their mineral compositions but high concentrations of calcium, magnesium, sodium, potassium and iron were obtained. Variations in mineral compositions of cereals from different locations were observed by Adeyeye and Ajewole[7] and were attributed to variation in soil type and/or weather condition. Ekpenyong[14] observed that there was differential accumulation in the mineral levels of maize varieties even with soil of similar fertility and attributed this to some genetic factors. Table 4 shows the results obtained from this work for some elements in comparison with what are in the literature for some generic samples. While the results are comparable for the phosphorus content, wide differences particularly for calcium are observed. The differences are higher than can be accounted for merely by soil fertility or use of fertilizer, variation in soil type and weather condition. Although there are also variations in some other elements contents, that of calcium attracts the most attention. It can then be generalized at this stage that the combined phosphate and phytate naturally present in the samples interfered seriously in the absorption behavior of calcium and hence very little of the latter could be determined in the flame. Before now, no sample preparation technique has been described for the accurate determination of calcium in cereals and results earlier published could be misleading. This lapse has been overcome in this report. After this, whatever other differences in the results are observed in Table 4 can be attributed to the factors listed earlier in addition to variety.

CONCLUSION

The results of this study have shown that better results can be obtained in the determination of the calcium content of cereals if strontium is incorporated in the sample matrix prior to analysis. Information obtained from this work can be very helpful in feed formulation since cereal grains are often used as important components of baby feeds, some adult beverages and in animal feed formulations. Accurate determination of calcium in cereals will help nutritionists in accurate estimation of daily aggregate allowances from different sources for meeting daily human requirements.

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