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Analytical Methods Section

Rapid determination of calcium, magnesium, iron and zinc in flours using flow injection flame atomic absorption spectrometry for slurry atomization

P. Viñas, N. Campillo, I. López García & M. Hernández Córdoba*

Department of Analytical Chemistry, Faculty of Chemistry, University of Murcia, E-30071-Murcia, Spain

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A procedure is described for the rapid determination of calcium, magnesium, iron and zinc in flours obtained from several grains and legumes (wheat, whole meal, maize, barley, rice, rye, vetch, soy bean and field bean) using flame atomic absorption spectrometry. Suspensions up to 3% m/V from the ground samples are directly introduced into the spectrometer using a simple flow injection manifold. Calibration is performed using aqueous standard solutions of the determinands. The results obtained with this procedure agree with those obtained by conventional dry-ashing followed by dissolution in acids. Results for two standard reference materials are also given.

INTRODUCTION

One drawback of atomic absorption spectrometry (AAS) in the analysis of foods is that solid samples must be dissolved to obtain solutions suitable for determination. This dissolution step is frequently tedious and involves a considerable expenditure of time and reagents. One alternative to the dissolution-based procedures is the direct use of the solid sample in such a manner that it can be introduced into the atomizer in a safe and reproducible way. The use of solid samples in AAS has been reviewed by several authors (Langmyhr & Wibetoe, 1985; Bendicho & Loos-Vollebregt, 1991) and can be carried out either by direct solid sampling or by sampling of suspensions. The use of suspensions prepared from samples with a fine particle size appears to be a reliable approach, as such slurries can be easily handled, in similar ways to liquid samples. Most of the papers related to foods or biological materials to date have dealt with electrothermal atomization of the slurries (Stephen *et al.*, 1985, 1987; Ebdon & Lechotycky, 1986; Fagioli *et al.*, 1986; Ebdon & Evans, 1987; Carrión *et al.*, 1988; Miller-Ihli, 1988; Lynch & Littlejohn, 1989; Kunwar *et al.*, 1990; Hernández Córdoba & López García, 1991), because particle size is less critical if this atomization mode is used. On the other hand, the introduction of the slurry into a flame has received less attention because low particle size is more important and there is a serious risk of the nebulizer clogging when the slurry is being aspirated. Additional problems can arise from incomplete atomization of the determinands or from matrix effects and a number of different approaches have been proposed to overcome these difficulties (Fagioli & Landi, 1983; Fagioli *et al.*, 1983; Wichman *et al.*, 1986; Carrión *et al.*, 1987).

The aim of the present paper is to describe a suspension-based AAS procedure for directly measuring the calcium, magnesium, iron and zinc contents in industrial flours obtained from several grains and legumes. To avoid nebulizer clogging, the slurry is supplied to the nebulizer in a discrete way, using a simple flow injection (FI) manifold. The procedure is rapid, avoids the sample dissolution step and affords results that agree with those obtained by using conventional dry-ashing and dissolution in acid.

MATERIALS AND METHODS

Apparatus and reagents

A Perkin-Elmer Model 1100B atomic absorption spectrometer with deuterium-arc background correction was used. Measurements were performed at 422·7, 285·2, 248·3 and 213·9 nm for calcium, magnesium, iron and zinc, respectively, using conventional hollow cathode lamps. Air-acetylene flames were used exclusively. The flow injection (FI) manifold shown in Fig. 1 was used. A Gilson Minipuls HP4 peristaltic pump was used together with an Omnifit injection valve. Sample

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* To whom correspondence should be addressed.

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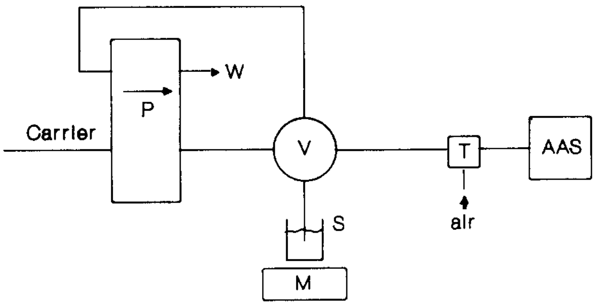

Fig. 1. Flow injection manifold. P, Peristaltic pump; V, injection valve, sample loop, 150-μl; S, sample reservoir; M, magnetic stirrer; T, T-piece for air compensation.

loops as well as all connecting lines were made of 0·8 mm i.d. polytetrafluoroethylene tubing. As indicated elsewhere (López García *et al.*, 1987, 1991), a three-way connector was included in the manifold to provide an inlet for air and aid the fragmentation of the sample plug. This T-piece allows air-compensation for the difference between the nebulizer uptake rate and the pumping flow rate, thus acting as a prenebulizer. The distance between the injection valve and the T-piece must be as short as possible.

Stock solutions (1000 μg ml^{-1}) of the four determinands were prepared and diluted as necessary to obtain working standards.

Procedures

Samples of barley, rye, field bean (*Vicia faba*), vetch, whole meal, rice, maize and soy bean were obtained as powders (flours) from an industrial mill. The samples of barley, rye, field bean, vetch and whole meal included remains of extraneous plant tissues and so they were sieved before analysing, through a 100 μm sieve, to remove larger particles. Samples of ordinary wheat flour were purchased in a supermarket and were not submitted to further sieving. To report the results on the dry weight basis, samples were dried at 65°C overnight or, alternatively, a correction for the moisture content was applied.

For the slurry procedure, the samples were ground in a ball mill for 30 min. Amounts of ground samples in the 0·02–0·75 g range (depending on the determinand and on its level in the sample being studied) were slurried in 25 ml of suspending solution. The suspensions were sonicated for 10 min and magnetically stirred for 10 min. While the slurry was being continuously stirred, the sample loop was filled, the plug injected into the FI manifold and the height of the transitory signal obtained. Calibration was performed by injecting aqueous solutions of the determinands under the same experimental conditions. A summary of the optimized experimental conditions is given in Table 1.

For comparison purposes, fractions of the samples were ashed at 500°C for 5 h. The ashes were treated with a mixture of nitric and hydrochloric acids and the solution finally diluted up to 100 ml. The contents of calcium, magnesium, zinc and iron were then determined by AAS in the conventional aspiration manner, using the conditions recommended by the manufacturer.

RESULTS AND DISCUSSION

The essential condition for suspension-AAS procedures to be used is a sufficiently small particle size. To achieve a convenient sample comminution, all the samples were submitted to grinding in a ball mill for 30 min. To study the stability of suspensions, a 2% slurry was prepared from wheat flour and magnetically stirred for 10 min. Stirring was then stopped, aliquots were injected into the FI manifold at 1 min intervals and the peak heights were obtained. The experiments were then repeated using suspensions prepared in the presence of various amounts of Triton X-100 or hexametaphosphate. It was shown that these agents did not significantly improve the stability and so, for the rest of the experiments, the suspensions were prepared without stabilizing agents and the sample loop was filled while the suspensions were being continuously stirred.

The spectrometer operating conditions, such as air and acetylene flow rates and the height of the burner, were optimized for the measurement of calcium using a 2% suspension prepared from commercial wheat flour. Optimal values were found to be the same as those for aqueous standards of calcium. These experiments were repeated for magnesium, iron and zinc and identical results were obtained. Thus, for the remaining experiments, the instrumental conditions were adjusted to be 1·6, 1·4, 1·6 and 1·3 litre min^{-1} of acetylene for Ca, Mg, Fe and Zn, respectively. The burner position under the beam of the hollow cathode lamp was 2 mm for all the elements.

Optimization of the loop size and the pumping rate was a compromise between sensitivity, carrier consumption and sampling frequency. The effect of both sample loop size and pumping rate was studied using

Table 1. Optimized experimental conditions

Element	Carrier	Suspending solution	Flow rate (ml min^{-1})	Sample loop (μl)
Ca	1% La(III) + 1% HCl	1% La(III) + 1% HCl	1·4	150
Mg	1% La(III) + 1% HCl	1% La(III) + 1% HCl	1·4	150
Fe	H_2O	4% HCl	1·8	150
Zn	1% HCl	1% HCl	1·4	150

Table 2. Statistical parameters of the proposed method

Parameter	Calcium	Magnesium	Iron	Zinc
RSD[a] (%) (n=10)	2·9 (230·5)	0·9 (642·8)	5·1(38·1)	3·9 (18·7)
Detection limit[b] μg g^{-1}	6·3	0·2	1·6	1·3
Determination limit[b] μg g^{-1}	21·2	0·8	5·3	4·4

[a] Values in brackets are the contents of the metal in the sample used in μg g^{-1}.
[b] Values calculated for a 3% suspension.

Table 3. Slopes of the standard additions calibration graphs

Medium	Slope			
	Calcium	Magnesium	Iron	Zinc
Slurry[a]	0·0198 ± 0·002	0·2079 ± 0·016	0·0143 ± 0·001	0·0694 ± 0·002
Aqueous standard[b]	0·0211 ± 0·001	0·2008 ± 0·004	0·0149 ± 0·001	0·0601 ± 0·004

[a] Mean ± SD (n = 6).
[b] Mean ± SD (n = 6).

four different loops with 35, 150, 200 and 300 μl volumes at different pumping rates in the 1–4 ml min^{-1} range. There was no significant increase in peak height when both increased beyond 150 μl and 2 ml min^{-1}, respectively. Greater sizes in the sample loop are not recommended because of the increased risk of the nebulizer or the FI connections clogging while the sample plug is being introduced into the atomizer. Optimal values for these variables are summarized in Table 1.

A practical drawback of the methodology here reported is the risk of clogging in the nebulizer or in the connections of the FI manifold. This is related to the particle size and with the percentage solids in the suspensions. The study of the particle size distribution was carried out with a flour sample ground for 5, 10 and 30 min periods. For each time period, samples were sieved through 30–250 μm sieves and weighed. Dry sieving was not adequate due to the formation of particle agglomerates. However, when wet sieving was applied, it was proved that almost all the sample (98%) passed through a 100 μm sieve, 21% of the particles were in the 30–50 μm range, and a fraction of 52% had a particle size less than 30 μm. These data were obtained using a 30 min grinding period. Next, the maximum suspension percentage possible which still avoided clogging was studied. Suspensions up to 4% of commercial wheat flour were tried and it was proved that when the percentage exceeded 3·5% the system frequently clogged.

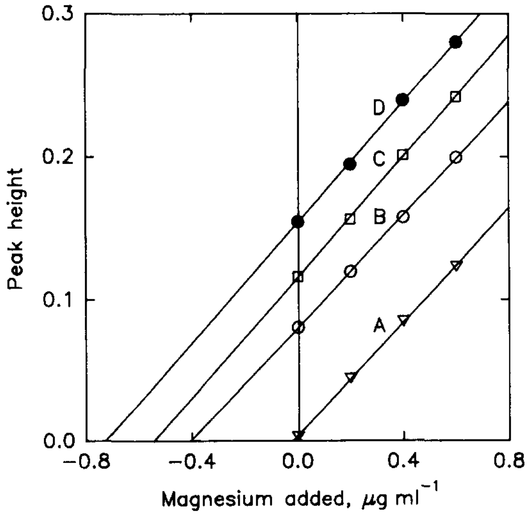

Fig. 2. Standard additions calibration graphs for the determination of magnesium in flours. A, Aqueous magnesium; B, 0·1% slurry from wheat flour; C, 0·08% slurry from rice; D, 0·05% slurry from vetch.

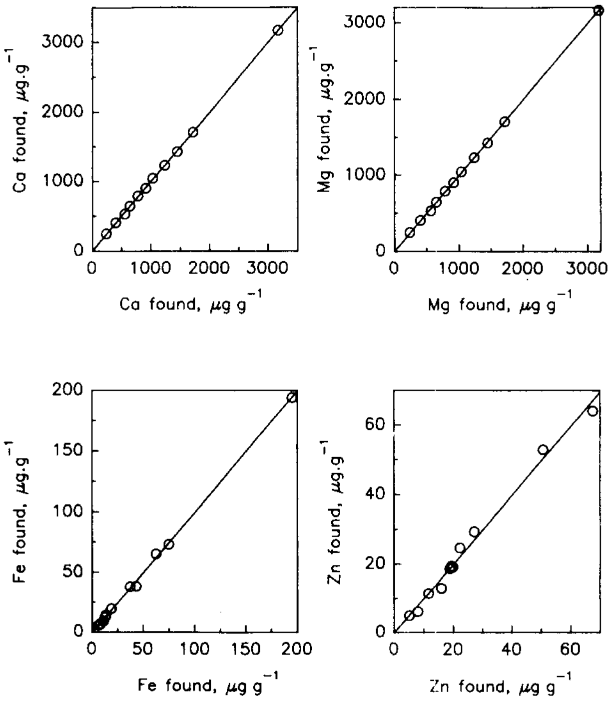

Fig. 3. Correlation graphs. Values on abscissae were obtained by dry-ashing and acid dissolution. Values on ordinate were obtained by the slurry procedure. Solid line indicates 100% correlation.

Table 4. Results for determination of Ca, Mg, Fe and Zn in flours by the slurry and dry-ashing procedures

Sample	Content (μg g^{-1})							
	Calcium		Magnesium		Iron		Zinc	
	A	B	A	B	A	B	A	B
Wheat	230·5	225·9	246·3	237·6	6·7	8·1	4·9	5·1
Whole meal	497·9	503·3	899·4	914·0	12·5	12·8	13·0	16·0
Rice	229·1	235·7	642·8	644·8	19·6	18·8	18·7	18·8
Field bean	3 285·0	3 283·0	1 426·0	1 444·0	65·2	62·5	24·7	22·2
Vetch	3 088·0	3 095·0	1 709·0	1 714·0	73·2	74·8	52·9	50·6
Barley	2 784·0	2 783·0	1 042·0	1 030·0	38·5	43·2	6·1	8·0
Rye	631·0	631·4	787·4	779·4	9·0	11·1	19·1	19·8
Maize	123·2	125·3	1 230·0	1 232·0	38·1	37·3	29·4	27·1
Soy bean	3 284·0	3 295·0	3 165·0	3 172·0	194·0	195·0	64·1	67·5

A, Slurry procedure.
B, Dry-ashing and acid-dissolution procedure.

Table 5. Comparison of the slurry and dry-ashing procedures

Metal	Statistical test				
	Wilcoxon *T*-test min(T+,T−)[a]	LSR[b] ($y = a + bx$)			
		$a \pm ts_a$	$b \pm ts_b$	t_a[c]	t_b[c]
Ca	15	−1·57 ± 5·51	0·9994 ± 0·0021	0·59	0·27
Mg	24	−1·44 ± 16·1	0·9976 ± 0·0120	0·03	0·42
Fe	20	−0·62 ± 3·02	0·9999 ± 0·0250	1·07	0·01
Zn	25	−0·08 ± 2·42	0·9949 ± 0·0814	0·07	0·14

[a] Tabulated value $T = 11$ for 95% significance level and $n = 11$.
[b] Method of least-squares fitting.
[c] Tabulated value of *t*-Student; $t = 2{\cdot}262$ for 95% significance level and $n = 9$.

Thus, from a practical point of view, suspensions more concentrated than 3% are not recommended, unless a more effective way of lowering the particle size is used.

The precision, expressed as the RSD, for 10 analyses of different slurries for the four determinands studied is shown in Table 2. Results for both the detection and the determination limits were calculated according to the criteria $\bar{x} \pm 3$SD and $\bar{x} \pm 10$SD, respectively, where $\bar{x}$ was the mean of 10 replicate determinations at the blank level and SD the corresponding standard deviation.

To select a suitable calibration procedure, standard addition calibration graphs were obtained for the four determinands using slurries prepared from the nine samples studied. Figure 2 shows the results obtained in the determination of magnesium content of several samples by the proposed procedure. Slopes indicate the absence of matrix effect. As shown in Table 3, similar results were obtained for Ca, Fe and Zn.

To prove if calibration with aqueous solutions is possible, contents of Ca, Mg, Fe and Zn in the flour samples were evaluated by extrapolation of the standard addition graphs. Results obtained are shown in Table 4.

The accuracy was also determined comparing the results obtained by the suspension procedure with those obtained by the conventional dry-ashing procedure indicated in the Materials and Methods section. As can be seen in Table 4, there was agreement between the two analytical approaches as deduced from the Wilcoxon *T*-test and the least-squares fitting. Statistical results are shown in Table 5 and indicate no significant differences between both procedures at the 95% confidence level. When applying the Wilcoxon *T*-test, the value of min ($T+$, $T-$) is always higher than the *T* value tabulated ($T = 11$). Application of the least-squares fitting led to experimental values of *a* (intercept on the ordinate) and *b* (slope) close enough to 0 and 1, respectively. The Student *t*-distribution for these parameters led to total correlation at the 95% confidence level. Figure 3 shows correlation graphs for Ca, Mg, Fe and Zn. As can be seen, almost 100% correlation between the values found using the suspension-based procedure reported here and the dry-ashing procedure was obtained.

In addition, to confirm the reliability of the analytical approach, two available standard reference materials were analyzed. Suspensions ranging from 1 to 3% for the measurement of Ca, Fe and Zn, and 0·2% for the determination of Mg were prepared. The results, which are shown in Table 6, were in agreement with those certified, although they were slightly low for the iron content in rice flour, which is close to the determination limit, and for the magnesium content in the same sample.

Table 6. Results for standard reference materials

Sample		Element			
		Calcium (μg g^{-1})	Magnesium (μg g^{-1})	Iron (μg g^{-1})	Zinc (μg g^{-1})
SRM 1567a (wheat flour)	Certified	191 ± 4	400 ± 20	14·1 ± 0·5	11·6 ± 0·4
	Found*	188 ± 6	405 ± 7	13·1 ± 1·0	11·0 ± 0·5
SRM 1568a (rice flour)	Certified	118 ± 6	560 ± 20	7·4 ± 0·9	19·4 ± 0·5
	Found*	119 ± 3	540 ± 10	6·8 ± 1·6	19·3 ± 0·5

* Mean ± SD ($n = 3$).

CONCLUSION

The results presented here prove that suspensions of plant material can be introduced, in a reliable manner, into the flame of an atomic absorption spectrometer by using a simple flow injection manifold. The discrete nature of the FI sample introduction, the rinsing action of the carrier stream and the inclusion in the manifold of a three-way connector with a tip open to the air are important factors which greatly minimize the possibility of the system clogging.

It is important to realize that this is not a general methodology and the success of the approach is related to particle size and the physical characteristics of the material being suspended. Taking this into account, the approach studied here can be of interest to those interested in obtaining analytical data on the metal content of vegetables, although specific studies are needed, depending on the characteristics of the sample and the level of each determinand.

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