Graphite-Furnace Atomic Absorption Spectrometric Method for Direct Determination of Iron and Zinc in Solid Rice Samples

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A method for the direct determination of iron and zinc traces in solid rice samples using graphite-furnace atomic absorption spectrometry is proposed. Samples are placed in a graphite tube with a platform, and are determined from integrated absorbance measurements that are interpolated on calibration graphs obtained from aqueous standards of the two metals. The working conditions used allow iron and zinc to be determined at concentrations down to 10 µg g⁻¹ (0.001%) in solid rice samples with a relative standard deviation of 6 - 10%.

Keywords Direct analysis, solid sample, rice analysis, iron determination, zinc determination, electrothermal atomic absorption spectrometry

Mineral substances in foods are not only physiologically significant, but are also activators or inhibitors for enzyme-catalysis processes, and are frequently responsible for food texture and flavor.

The iron demand of the human body is about 1 - 2.8 mg d⁻¹, which can be met by a food intake supplying 5 - 28 mg of iron daily. Individuals with high iron demand are commonly placed on diets containing abundant cereals (55 - 130 mg kg⁻¹) or rice (6 - 26 mg kg⁻¹). To food technologists, iron is frequently detrimental because it acts as a catalyst for fat oxidation.

There are strong reasons to consider zinc to be an "unassuming nutrient". In fact, this metal is known to take part in more than 200 enzymatic processes, some of which are crucial to human health; however, zinc deficiencies have even more implications than those involved in enzymatic reactions can account for. In the absence of an adequate dietary supply of this metal, serious health problems arise. Zinc occurs widely in foods and beverages; in some, such as commodities of vegetable origin, it is present at very low concentrations. Although cereals are in principle suitable sources of zinc, they lose some of the metal during milling. The zinc content in rice ranges from 5 to 25 mg kg⁻¹.

The iron and zinc contents in foods, beverages and biological materials in general can be readily measured by using flame and electrothermal atomic absorption techniques. As a rule, an air-acetylene flame is appropriate for this purpose, since it is free of the type interference that is typically posed by decomposed organic matter. A sample can be processed by wet digestion in an acid oxidant solution, or by dry ashing and subsequent acid dissolution. The formation and atomization of slurries is another competent choice for the determination of both metals in foods.

The direct determination of metals in solid samples using graphite-furnace atomic absorption spectrometry (GFAAS) has the advantage that it avoids any sample pretreatment step, which results in substantial time and labor savings, and also avoids potential problems arising from contamination or analyte loss. In addition, because no sample dilution is required, it is highly sensitive. On the other hand, sample handling is somewhat cumbersome, particularly introduction into a conventional atomizer. The results are usually scarcely precise, which is frequently ascribed to sample heterogeneity, the relative standard deviation (RSD) frequently being close to 10%. Additional shortcomings of this technique include a shortage of appropriate calibration standards and the presence of matrix interference. Although the latter is not always encountered, by using samples whose matrix can be largely removed in the calcination step, calibration against aqueous solutions is possible, and matrix effects after calcination are either nil or can be readily overcome by using a deuterium lamp.

Iron has been directly analyzed by GFAAS in solid samples, both biological and geological, with no special problems other than those derived from the typically excessive sensitivity of this technique.

Zinc has also been determined in biological samples, with calibration against aqueous solutions in only one instance. The metal has also been deter-
mined directly in other types of matrices, including steel and alloys\(^{16}\) (which reportedly involving double peaks)\(^{17}\), uranium oxide (using graphite as a matrix modifier)\(^{18}\) and coal.\(^{19}\)

In previous work\(^{10,20-22}\), we found that non-pulverulent samples can readily be introduced into an atomizer, provided that the matrix is removed in the calcination step; we also found calibration against aqueous solutions to provide satisfactory results if a deuterium corrector is used. In this work we developed a simple, fast method for determining iron and zinc in rice that can be used in those cases where expeditiousness is favored over precision.

**Experimental**

**Apparatus**

The Perkin-Elmer 3030 atomic absorption spectrometer was connected to an HG-400 graphite furnace equipped with a temperature programmer and an AS40 autosampler, also from Perkin-Elmer. Pyrolytic-coated graphite tubes with a platform were used; their hole was mechanically enlarged to a diameter of about 4 mm. A deuterium lamp was used for a background correction. Hollow-cathode lamps employed were purchased from Perkin-Elmer.

**Samples and reagents**

A standard containing 1000 µg ml\(^{-1}\) iron was prepared by weighing to within 0.0001 g approximately 1.00 g of metal iron and dissolving it in 50 ml of 1+1 HNO\(_3\). The solution was made to 11 with de-ionized water. Another standard containing 1000 µg ml\(^{-1}\) zinc was also made by weighing to within 0.0001 g about 1.00 g of metal, dissolving it in a minimum volume of 1+1 HCl and making to 11 with 1% (v/v) HCl.

All of the solutions were made from analytical reagent grade chemicals. Additional dilute solutions of the metals were prepared by diluting the standards with distilled water prior to use.

Two commercially available samples of rice from Herba S.A. with nominal iron (samples 1 and 2) and zinc contents (sample 2) of ca. 10 µg g\(^{-1}\) (0.0010%) and 11 µg g\(^{-1}\) (0.0011%), respectively, were used. The samples also contained calcium (200 µg g\(^{-1}\)), phosphorus (1000 – 1500 µg g\(^{-1}\)), sodium (4 – 6 µg g\(^{-1}\)), potassium (85 – 95 µg g\(^{-1}\)), protein (7.3%) and carbohydrates (70%).

**Procedure for the determination in dissolved samples**

Sample 1 was treated by acid digestion with HNO\(_3\) for 3 h and sample 2 by calcination at 800°C for 8 h and subsequent dissolution in aqua regia in accordance with the AOAC recommended procedure.\(^{23}\) The metals were determined by GFAAS, with calibration against aqueous standards. The operating conditions used are summarized in Table 1. Iron determinations entailed using a blank containing the reagents, since their impurities gave appreciable absorbance readings that led to overestimated values.

**Procedure for the determination in solid samples**

Rice grains were held with stainless-steel pincers for excision with scissors of the same material, and weight to within 1X10\(^{-5}\) g. The samples were suspended in water drops and directly placed in a pyrolytic graphite tube with a platform with the aid of a conventional micropipette, the tip of which was previously trimmed in such a way that its inner diameter would exceed that of the sample. The sample mass used ranged from 0.5 to 3 mg.

Calibration graphs were constructed from iron and zinc standards containing the concentrations given in Table 1. For the determinations, an amount of solid rice sample having 0.75 – 2.25 mg (Fe) and 1.75 – 2.25 mg (Zn) was suspended in a water drop and placed in a graphite tube. The iron and zinc contents were determined by interpolation on the calibration graphs. The result for each determination was taken to be the median of 5 consecutive measurements.

Solutions and real samples were atomized electrothermally, and the atomic absorption (integrated absorbance) of iron and zinc was measured under the operating

| Table 1 Instrument parameters used in the determination of iron and zinc by GFAAS |
|------------------|------------------|
|                  | Iron             | Zinc            |
| Wavelength/nm    | 248.3            | 307.6           |
| Intensity/mA     | 20               | 25              |
| Slit width/nm    | 0.7              | 2.0             |
| Gas flow/ml min\(^{-1}\) | 300             | No              |
| Working range/ng | 0 – 40           | 0 – 80          |
| Injected volume/µl | 20              | 20              |
| Sample mass/mg   | 0.75 – 3         | 1.25 – 3        |
| Drying step      |                  |                 |
| Temperature/°C   | 120              | 120             |
| Ramp/s           | 20               | 20              |
| Hold time/s      | 30               | 30              |
| Charring step    |                  |                 |
| Temperature/°C   | 550 – 1000       | 900             |
| Ramp/s           | 30 – 10          | 20              |
| Hold time/s      | 60 – 30          | 20              |
| Atomization step |                  |                 |
| Temperature/°C   | 2500             | 2300            |
| Ramp/s           | 0                | 0               |
| Hold time/s      | 3                | 2               |
| Cleaning step    |                  |                 |
| Temperature/°C   | 2700             | 2700            |
| Ramp/s           | 1                | 1               |
| Hold time/s      | 3                | 3               |
Results and Discussion

Sample introduction

With the aim of using conventional equipment to perform the determinations, in previous work\textsuperscript{10,20-22} the sample was introduced into the pyrolytic tube via an inverted paper cone with a pierced apex that was inserted into the enlarged hole of the tube. Despite its simplicity, the procedure had the disadvantage that the position where the sample was placed was irreproducible.

Non-pulverulent samples can be more readily inserted by using a conventional micro pipette, as illustrated in Fig. 1. The sample is suspended in a water drop and the micropipette tip is trimmed to an internal diameter exceeding that of the sample. The solid is thus aspirated with the drop and then swept by the water into the tube or platform as the micropipette is unloaded. This procedure is simpler and the sample positioning quite reproducible.

Selection of the working wavelength

GFAAS is frequently too sensitive for direct determinations of solid samples.\textsuperscript{24,25} The problem is commonly solved by using less sensitive lines and maintaining the argon flow during the atomization step.

In previous work\textsuperscript{10} we assessed various procedures for the determination of iron in solid samples. Based on the results, we chose to use the 248.3 nm line and to maintain argon flow during atomization.

The only two lines that provided appreciable signals for zinc were those at 213.8 and 307.6 nm. The former is the most sensitive line for this element, and would have been appropriate for the typical zinc contents in rice; however, it could not be used, since it was strongly interfered by an iron line (see Fig. 2). The interference was of spectral nature (213.856 nm for zinc and 213.859 nm for Fe).\textsuperscript{26} We thus had to use the line at 307.6 nm, which had previously been employed by other authors\textsuperscript{13-16}, but with somewhat higher zinc contents than those in our samples. Obviously, the argon flow was stopped during the atomization step. The best results were obtained at a monochromator slit width of 2.0 nm.

Optimization of the atomization conditions

Calcination is the most crucial step for a correct development of the process, since it must remove as much matrix as possible with no appreciable analyte loss. The matrix-removal process can be visually observed (as thick smoke coming out of the atomizer). Because we had atomized solid-food samples directly from the tube walls in previous work\textsuperscript{10,21}, we tested the same conditions, which, however, proved to be unsatisfactory. In fact, however gentle load the calcination ramp, the sample tended to crackle and move inside the tube, which gave rise to poorly reproducible results. We therefore chose to use a L'vov platform, which avoided the above-described problems.

The calcination temperature used for the determination of iron had to exceed 600°C; otherwise matrix removal could be incomplete and a non-specific absorption peak preceding or overlapping that for iron could be obtained in the atomization step (see Fig. 3a). On the other hand, atomization temperatures above 1000°C led to iron losses.

Although matrix removal was virtually complete at calcination temperatures from 600 to 1000°C, a double peak was obtained even at very long ramp and hold times (see Fig. 3b). Avoiding this double peak entailed using two calcination steps: one at 550°C and the other at 1000°C. Under these conditions, a single, well-defined peak, such as that shown in Fig. 3c, was obtained.

The calcination temperature for the determination of zinc also had to be higher than 600°C; otherwise, non-specific absorption was observed as a result of incomplete matrix removal (Fig. 4a). A calcination temperature of 900°C held for 20 s provided a well-defined peak and inappreciable non-specific absorption (Fig. 4b).

All other operating conditions proved to be less critical for both metals.
Calibration

As can be seen in Fig. 5, the signals for iron in a solution and a solid sample obtained under the optimal conditions were very similar; however, the solution signal appeared sooner than the solid sample signal. This was also the case with zinc, even though the signals appeared at virtually the same time. The results confirmed the advisability of using calibrations with aqueous solutions of the two metals. The equations for the calibration graphs, fitted by least-squares regression were

\[ Q_A = 0.0266 \text{ ng Fe} + 0.003 \]

and

\[ Q_A = 0.0010 \text{ ng Zn} + 0.009, \]

where \( Q_A \) denotes the integrated absorbance, and the correlation coefficient was close to 0.999 in both cases.

Analytical results

After the optimal instrumental conditions for the measurements were established, the analytical behavior of iron and zinc in the samples was studied. To this end, a total of 71 measurements of iron in sample 1 on 8 different days, 48 measurements of Fe in sample 2 on 4 different days, and 97 measurements of Zn in sample 2 on 5 different dates were made. The mean values thus obtained (viz. 11.7 \( \mu \text{g} \text{ g}^{-1} \) Fe in sample 1, 11.1 \( \mu \text{g} \text{ g}^{-1} \) Fe in sample 2 and 11.2 \( \mu \text{g} \text{ g}^{-1} \) Zn in sample 2) were quite consistent with those measured in the sample solution (Table 2); however, the precision was rather poor (RSD>30%), as shown in the graph of Fig. 6a.

Since previous work on other elements and matrices\(^{10,20-22}\) revealed that the results obtained under our working conditions were frequently dependent on the amount of sample used, we studied its effect on the basis of the above-described results. For this purpose, the results were grouped according to the sample mass ranges in 0.25 mg steps, and the median for each group was calculated. Using the median instead of the mean minimized the influence of the potential outliers\(^{27-29}\) arising from sample heterogeneity, the presence of nuggets\(^{30}\) and other sources.\(^{31}\) As can be seen from
Fig. 7, the results were typical for our working conditions: small masses gave rise to underestimated values and large masses gave overestimated results. The optimal mass ranges for Fe and Zn were 0.75 - 2.25 and 1.75 - 2.25 mg, respectively.

Using more measurements is known to increase the precision of a determination;\textsuperscript{32} however, the improvement is negligible beyond a given number. In order to determine the optimal number of measurements for our determination, the initial results were grouped in series consisting of 1 - 7 values; the median for each series was taken as the value for one determination; the mean and relative standard deviation for all the medians were calculated. The mean was found to vary little; however, as can be seen in Fig. 8, the RSD decreased markedly with increasing number of measurements. With 5 measurements, the RSD for both iron and zinc was more than acceptable for the technique used, and more measurements resulted in no significant improvements.

Table 2  Summary of the results obtained in the determination of iron and zinc in solid samples of rice by GFAAS

| Parameter                        | Sample 1 | | | Sample 2 | | | |
|---------------------------------|----------|----------|----------|----------|----------|----------|
|                                 | Solid\textsuperscript{a} | Solution\textsuperscript{b} | Solid\textsuperscript{a} | Solution\textsuperscript{b} | Solid\textsuperscript{a} | Solution\textsuperscript{b} |
| Number of determination         | 7        | 5        | 7        | 5        | 10       | 5        |
| Average value/µg g\textsuperscript{-1} | 10.8     | 10.2     | 10.9     | 10.8     | 10.8     | 11.2     |
| Range/µg g\textsuperscript{-1}  | 8.9 - 12.0 | 8.6 - 11.5 | 9.5 - 12.2 | 9.3 - 12.2 | 9.4 - 12.0 | 10.5 - 12.2 |
| Standard deviation/µg g\textsuperscript{-1} | 1.0     | 1.1     | 1.1     | 1.3     | 0.9     | 0.7     |
| RSD, %                          | 9.6      | 11.2     | 9.9      | 11.7     | 8.1      | 6.0      |

a. Five replicates for each determination. b. Determined by GFAAS after acid attack (HNO\textsubscript{3}+H\textsubscript{2}SO\textsubscript{4}). c. Determined by GFAAS after ashing and dissolution in aqua regia.
**Determination of iron and zinc in rice samples**

After the optimal instrumental conditions and amount of sample to be used were established, iron was determined 7 times in samples 1 and 2 on three consecutive days, and zinc 10 times in sample 2 on four consecutive days. Each determination was carried out in quintuplicate and the iron or zinc content in each was calculated by interpolation of the calibration curves. The results are shown in Fig. 6b. The median for the 5 replicates of each determination was taken as the result (see Fig. 6c).

The results obtained in the direct determination of the two metals in the solid sample were compared with those provided by GFAAS following a conventional treatment of the samples. As can be seen from Table 2, there was good consistency between the two sets of results and with the manufacturer’s stated contents. Statistical tests at a confidence level of 95% revealed no significant differences between the results provided by the two methods as regards mean values (t=0.94 for Fe in sample 1, t=0.22 for Fe in sample 2, and t=0.05 for Zn) and precision (F=1.22 for Fe in sample 1, F=1.41 for Fe in sample 2, and F=2.08 for Zn).

**Throughput**

Since each determination of iron and zinc in a real sample of rice under the above-described optimal conditions was approximately 15 min, the throughput was about 4 samples h⁻¹.

In conclusion, the proposed method allows the straightforward, rapid determination of iron and zinc in rice. Based on the results, it should be equally applicable to other cereal samples with no substantial alteration. The accuracy and precision of the results, similar to those achieved by processing the sample conventionally, are adequate for using the method in control analyses. In broad terms, the method is a useful choice in those cases where expeditiousness is a priority criterion.

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**References**

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