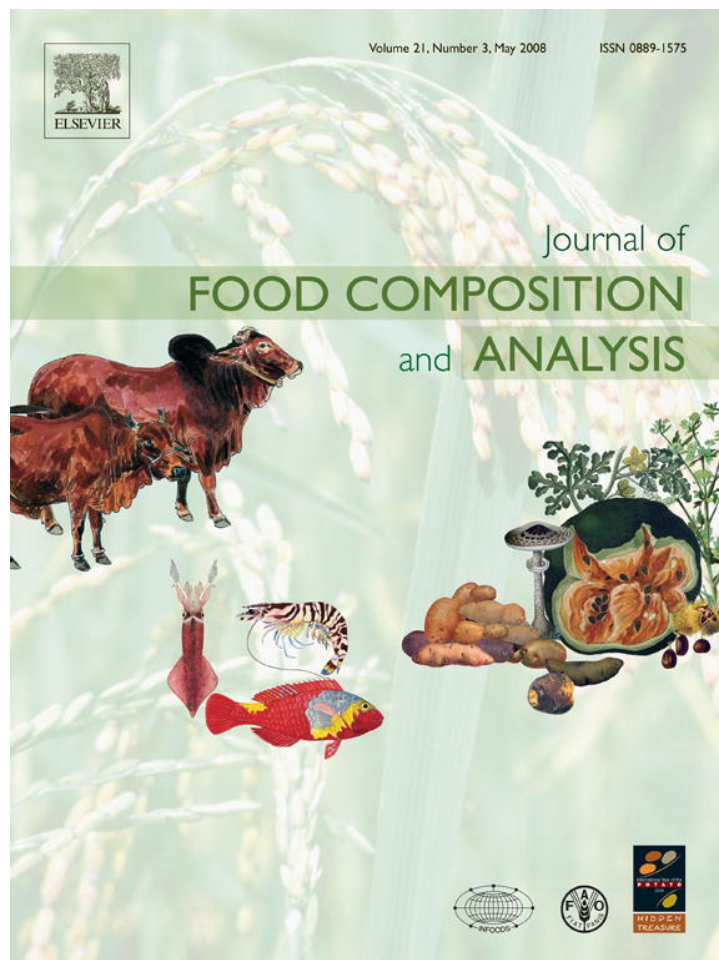


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Original Article

# Fast method for the determination of copper, manganese and iron in seafood samples

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## Abstract

A slurry sampling flame atomic absorption spectrometric method is proposed for the determination of copper, manganese and iron in oysters (*Crassostrea rhizophora*), clams (*Anomalocardia brasiliana*) and mussels (*Mytella guyanensis*; *Perna perna*). Optimization was carried out using univariate methodology involving the following variables: nature and concentration of the acid solution for slurry preparation, sonication time and sample mass. The optimized conditions were 80 mg of sample ground in a cryogenic mil, dilution using 1.0 mol L<sup>-1</sup> nitric/hydrochloric acid solution, sonication time of 30 min and a slurry volume of 10 mL. The calibration curves had been prepared using standards of copper, manganese and iron also in 1.0 mol L<sup>-1</sup> nitric/hydrochloric acid solution. This method allowed the determination of copper, manganese and iron by FAAS, with detection limits of 0.17, 0.09 and 0.46 µg g<sup>-1</sup>, respectively. The precision, expressed as relative standard deviation (RSD), are 3.0%, 2.9% and 3.8% (*n* = 10), for concentrations of copper, manganese and iron of 17, 22 and 719 µg g<sup>-1</sup>, respectively. The accuracy of the method was confirmed by analysis of the certified oyster tissue (NIST 1566b). The proposed method was applied for the determination of copper, manganese and iron in samples of oyster, clams and mussels, collected in Ubatuba and Todos os Santos Bay, Brazil. The obtained concentrations varied between 17.1 and 143.9 µg g<sup>-1</sup>, 17.9 and 29.7 µg g<sup>-1</sup> and 364 and 1388 µg g<sup>-1</sup>, respectively, for copper, manganese and iron. These values agree well with other data reported in the literature. The results showed no significant differences (*P* > 0.005) using the proposed method with those obtained after complete digestion and determination by inductively coupled plasma-optical emission spectroscopy (ICP-OES).

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**Keywords:** Determination of copper manganese and iron; Cu; Mn; Fe; Slurry sampling; Fast sequential; Seafood samples; Oysters; *Crassostrea rhizophora*; Clams; *Anomalocardia brasiliana*; Mussels; *Mytella guyanensis*; *Perna perna*

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## 1. Introduction

Recently, several methods for direct analysis of complex matrices have been developed and many reviews have been published (Vale et al., 2006; Cal-Prieto et al., 2002; Matusiewicz, 2003; Ebdon et al., 1997; Santos and Nóbrega, 2006). Among these studies, the slurry sampling technique is one of the most cited methods. The

simplification of the sample pre-treatment step, the decrease of the operational work and analysis time, low risks of sample contamination and minimization of analyte losses caused by the pre-treatment steps are among the main advantages of this technique. Methods employing slurry sampling for analysis of seafood has been proposed for the determination of mercury (Moreda-Pineiro et al., 2002; Vinas et al., 2001), selenium (Mendez et al., 2001), total arsenic (Santos et al., 2000) and cadmium (Bermejo-Barrera et al., 2000), all using electrothermal atomic absorption spectrometry (ET AAS).

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Cryogenic grinding is a process carried out at low temperature with frozen samples, which allows a good sample homogenization, and yields more accurate results. This technique allows an efficient reduction of the size of particles. The combination of cryogenic grinding and slurry sampling has not been widely applied. Methods using this combination have been proposed for analysis of hair (Kamogawa et al., 2001), food (Santos et al., 2002), human teeth (Santos et al., 2003), seeds and plant reference materials (Engelsen and Wibetoe, 2000), all these using ET AAS as analytical technique.

Several strategies have been performed in order to allow the application of the atomic absorption spectrometry as multi-element technique (Welz et al., 2003; Welz, 2005). Instruments have been developed for simultaneous determination using graphite furnace atomic absorption spectrometry (Hammond et al., 1998), flame atomic absorption spectrometry (FAAS) (Thiem et al., 1993) and W-coil atomic absorption spectrometry (Salido and Jones, 1999). Recently, a spectrometer for sequential multi-element determinations by FAAS was also proposed (Projahn et al., 2004). The sequential mode of this instrument allows the measurement of the absorbance in a sequence of wavelengths using a scan monochromator and software to change the operational conditions in short time intervals (Projahn et al., 2004). Methods using this equipment have been previously applied (Wang et al., 2003; Amorim and Ferreira, 2005).

In this paper, a method for the determination of copper, manganese and iron in seafood samples (oysters, clams and mussels) has been proposed using slurry sampling after cryogenic grinding of samples. The analytical measure was established employing the sequential multi-element FAAS.

## 2. Experimental

### 2.1. Instrumentation

A Varian Model SpectrAA 220 FS (Mulgrave, Victoria, Australia) flame atomic absorption spectrometer with fast sequential module (FS-FAAS), equipped with a conventional system pneumatic nebulizer and nebulization chamber was used for the analysis. The multi-element copper, iron and manganese hollow cathode lamp was operated under a current of 10 mA. The wavelengths for manganese (279.5 nm), copper (324.8 nm) and iron (372.0 nm) were used with bandwidths of 0.2, 0.5 and 0.2 nm for manganese, copper and iron, respectively. The flame composition was acetylene ( $2.0 \text{ L min}^{-1}$ ) and air ( $13.5 \text{ L min}^{-1}$ ) and the burner height was adjusted to copper, the element with the smallest absorbance signal. The nebulizer aspiration flow rate was kept between 5.5 and  $6.0 \text{ mL min}^{-1}$ . The validation step was carried out employing an inductively coupled plasma optical emission spectrometer VISTA-PRO (Varian, Mulgrave, Australia). This instrument is equipped with a cyclonic spray chamber, a concentric nebulizer and a solid-state detector that allows measurements from 167 to

785 nm. The pre-optical system was purged with argon in order to enable readings below 180 nm. The polychromator was thermostated at  $34^\circ\text{C}$  and purged with argon. An end-on gas interface was used to minimize interferences caused by self-absorption and recombination, to provide a wide linear dynamic range and low background. The emission lines used were 324.754, 238.204, 257.610 nm for copper, iron and manganese, respectively. The particle size was measured using a Shimadzu (Kioto, Japan) Superscan SS-550 scanning electron microscope. An Ultrasonic Benchtop Cleaner VWR Model 75 D (Cortland, NY, USA) was used for slurry preparation. The sample digestion was carried out in a hot-plate QUIMIS model Q 313/22 Diadema, São Paulo, SP). A SPEX CertiPrep Model 6750 freezer/mill (Metuchen, NJ, USA), with a self-containing liquid nitrogen bath was used to grind samples.

### 2.2. Reagents and samples

All reagents were of analytical grade unless otherwise stated. Ultrapure water was obtained from an EASYpure RF (Barnstedt, Dubuque, IA, USA). Suprapur nitric and hydrochloric acid (Merck, Darmstadt, Germany) were used. Laboratory glassware was kept overnight in 10% v/v nitric acid solution. Before use the glassware was rinsed with deionised water and dried in a dust free environment. Manganese, iron and copper solutions ( $100.0 \mu\text{g mL}^{-1}$ ) were prepared by diluting a  $1000 \mu\text{g mL}^{-1}$  solution (Merck) with a 1% (v/v) nitric acid solution. Nitric and hydrochloric acid solutions were prepared by dilution with water from the concentrated suprapur acids.

Samples of oysters (*Crassostrea rhizophora*), clams (*Anomalocardia brasiliiana*) and mussels (*Mytella guianensis* and *Perna perna*) were collected in Todos os Santos and Ubatuba Bay, Brazil. Samples of clams and mussels were collected in two sites, hereafter called A and B. Care was taken during all steps of sample collection and pre-treatment to avoid contamination. The certified reference material oyster tissue (NIST 1566b; National Institute of Standard and Technology) was used to check the accuracy.

The rice flour used for blank preparation for the determination of the analytical features was MAIZENA (Unilever Bestfoods Brazil Ltda, Garanhuns, Pernambuco, Brazil), commercially available in Brazilian markets.

### 2.3. Cryogenic grinding

A mass of 5 g of samples was inserted in a grinding vial, a polycarbonate cylinder supplied with two stainless steel plugs, immersed in liquid nitrogen and ground with an alternating magnetically driven steel impactor. Samples were precooled for 3 min and grounded for 2 min. Three grinding cycles, with a cooling step of 1 min between cycles, were applied to each sample, totaling 18 min.

#### 2.4. Slurry preparation

A mass of 80 mg (maximum particle size  $\cong 84 \mu\text{m}$ ) of powdered tissue was weighed accurately, transferred to a 10 ml volumetric flask, and the volume was made up using  $1.0 \text{ mol L}^{-1}$  nitric/hydrochloric acid solution. The slurries were homogenized for 30 min in an ultrasonic bath. Then, slurries were aspirated into the nebulizer for the determination of metals. Blanks were prepared in the same way as sample slurries, using rice flour, which presents metal concentrations lower than the quantification limit of the proposed method.

#### 2.5. Total digestion of seafood samples

A mass of 200 mg of samples was weighed in 125-mL erlenmeyer and 10 mL of concentrated nitric acid and 5 mL of 30% (v/v) hydrogen peroxide were added. Then, samples were heated up to a complete digestion. The solutions were left to cool down to room temperature, transferred to a calibrated flask and diluted to a final volume of 10 ml with  $1.0 \text{ mol L}^{-1}$  nitric/hydrochloric acid solution. Metals were determined in the final solution by inductively coupled plasma-optical emission spectroscopy (ICP-OES). All samples were analysed in triplicate.

### 3. Results and discussion

The method was optimized using univariate methodology. First, slurries were prepared using 1.0, 2.0, 3.0 and  $4.0 \text{ mol L}^{-1}$  solutions of nitric and hydrochloric acid. For copper and manganese the best results were obtained using nitric acid, whereas for iron, the hydrochloric acid showed more efficiency. As a result, slurries were prepared using acid solutions containing simultaneously nitric and hydrochloric acid in the concentrations of 0.5, 1.0 and  $1.5 \text{ mol L}^{-1}$ . For all metals high analytical signals were always achieved using  $1.0 \text{ mol L}^{-1}$  nitric acid/hydrochloric acid solution. Using nitric/hydrochloric acid solution  $0.5 \text{ mol L}^{-1}$  caused occasional blockage in the nebulization system of spectrometer. All these tests were carried out using sonication time of 20 min and a sample mass of 60 mg.

The effect of sonication time in the preparation of the slurries was studied in the range of 10–50 min, with interval of 10 min. Results demonstrated that the studied time range had no influence on the analytical signals obtained. However, when using 10 and 20 min of sonication there was an occasional obstruction of the nebulization system. Thus, a sonication time of 30 min was recommended for this method.

The effect of sample mass was also evaluated. Slurries were prepared using 40, 80, 120, 160 and 200 mg of sample, sonication time of 30 min was applied and a final volume of 10 mL using  $1.00 \text{ mol L}^{-1}$  nitric acid/hydrochloric acid solution. This experiment showed that there are linear relationships between sample mass and absorbance for

copper in the range of 0–120 mg, for manganese between 0 and 160 mg and for iron between 0 and 100 mg. Hence, for multi-element determinations the procedure recommends use of 80–100 mg of sample.

The process of extraction of the proposed method was investigated for all metals. Slurries were prepared using 80 mg of sample, dilution for 10 mL using  $1.0 \text{ mol L}^{-1}$  nitric/hydrochloric acid solution and sonication time of 30 min. Relationships of absorbencies obtained for slurries and for the liquid phase of the same slurries after centrifugation and filtration were used for evaluation. The extraction efficiency was 71% for iron, 85% for copper and 90% for manganese. These results demonstrated that in these conditions the extraction process was not complete for all studied metals.

The FS-FAAS system has a limitation. The burner position is fixed during the sequential determination (Projahn et al., 2004). The adjustment should be done by the element of the smallest sensitivity and/or lowest concentration in samples. In this work, the optimization was performed for copper, because of its lowest concentration.

#### 3.1. Analytical parameters

The calibration of the method was also investigated. The analytical curves were prepared using the standard calibration technique, with dilution of metal standards in  $1.00 \text{ mol L}^{-1}$  nitric/hydrochloric acid solution. Calibration curves using the analyte addition technique were prepared in presence of a seafood sample. All correlation coefficients were above 0.999. Equations of the analytical curves are presented in Table 1. It can be seen that results exhibited good similarity among the obtained slopes, demonstrating that the studied elements can be determined either using the standard addition method or the external calibration technique. Because of the simplicity of the procedure, it is

Table 1  
Equations of the analytical curves

Metal ion	Equations using the standard calibration technique	Equations using the standard addition technique
Copper	Abs = 0.0908.C <sub>Cu</sub> + 0.0066	Abs = 0.0950.C <sub>Cu</sub> + 0.0038
Manganese	Abs = 0.1707.C <sub>Mn</sub> + 0.0352	Abs = 0.1727.C <sub>Mn</sub> + 0.0296
Iron	Abs = 0.0148.C <sub>Fe</sub> + 0.0637	Abs = 0.0145.C <sub>Fe</sub> + 0.0552

Abs = absorbance.

Table 2  
Results of the certified oyster tissue sample (NIST 1566b)

Metal ion	Certified value ( $\mu\text{g g}^{-1}$ )	Proposed method <sup>a</sup> ( $\mu\text{g g}^{-1}$ )
Copper	71.6 $\pm$ 1.6	70.5 $\pm$ 1.8
Manganese	18.5 $\pm$ 0.2	17.6 $\pm$ 0.7
Iron	205.8 $\pm$ 6.8	206.4 $\pm$ 3.3

<sup>a</sup>All results are expressed as confidence intervals at 95% and  $n = 3$ .

Table 3  
Results of copper, manganese and iron in seafood samples using the proposed method

Sample	Determination of copper		Determination of manganese		Determination of iron	
	Proposed method	Digestion/ICP-OES	Proposed method	Digestion/ICP-OES	Proposed method	Digestion/ICP-OES
Mussel <sup>a</sup>	17.1 ± 0.9	17.4 ± 0.9	21.8 ± 0.6	22.1 ± 0.5	719 ± 1.9	720 ± 3.4
Mussel <sup>b</sup>	21.5 ± 1.2	22.3 ± 1.2	29.7 ± 1.1	30.9 ± 1.0	1388 ± 2.1	1388 ± 3.8
Mussel <sup>c</sup>	17.5 ± 1.0	18.0 ± 1.4	17.9 ± 1.2	17.3 ± 0.7	397 ± 1.5	396 ± 2.5
Clam <sup>d</sup>	19.8 ± 1.9	21.8 ± 1.0	26.4 ± 1.2	27.0 ± 1.2	507 ± 1.4	507 ± 2.9
Clam <sup>e</sup>	17.5 ± 2.2	19.4 ± 0.8	19.0 ± 1.1	19.6 ± 1.0	416 ± 2.6	418 ± 3.4
Oyster <sup>f</sup>	144 ± 1.1	142 ± 0.9	27.2 ± 0.7	26.7 ± 1.3	363 ± 1.3	364 ± 3.0

All the results are expressed as confidence intervals at 95% and  $n = 3$ .

<sup>a</sup> *Mytella guianensis*/A.

<sup>b</sup> *Mytella guianensis*/B.

<sup>c</sup> *Perna perna*.

<sup>d</sup> *Anomalocardia brasiliiana*/A.

<sup>e</sup> *Anomalocardia brasiliiana*/B.

<sup>f</sup> *Crassostrea rhizophorae*.

recommended the determination of all metals using the external calibration technique.

The detection limits were calculated as IUPAC recommendation (IUPAC-Analytical Chemistry Division, 1978), considering the standard deviations of the blanks, which were prepared as slurries using rice flour. Detection limits are 0.17, 0.09 and 0.46  $\mu\text{g g}^{-1}$  for copper, manganese and iron, respectively. The precision, expressed as relative standard deviation (RSD) are 3.0%, 2.9% and 3.8% ( $n = 10$ ), for concentrations of copper, manganese and iron of 17, 22 and 719  $\mu\text{g g}^{-1}$ , respectively. The accuracy was evaluated by analysis of the certified material oyster tissue (NIST 1566b). The results given in Table 2 confirm the accuracy of the proposed method.

### 3.2. Application

The proposed method was applied for the determination of copper, manganese and iron in six seafood samples (oyster, clams and mussels), collected in Ubatuba and Todos os Santos Bay, Brazil. The results obtained using the proposed method were compared with results obtained after open vessel acid digestion of samples and ICP-OES determination. The results are given in Table 3. The obtained concentrations of copper varied between 17.1 and 143.9  $\mu\text{g g}^{-1}$  for copper, 17.9 and 29.7  $\mu\text{g g}^{-1}$  for manganese, and 364.3 and 1388.4  $\mu\text{g g}^{-1}$  for iron. These values agree well with other data reported in the literature (Alfonso et al., 2005; Tuzen et al., 2007).

### 4. Conclusions

A combination of cryogenic grinding and slurry sampling allows a fast method for determination of copper, manganese and iron in seafood samples by FAAS. The low contamination risk and simple handling besides the possibility of standardization using aqueous reference solutions are desirable features of procedures for routine

applications. The slurry preparation is rapid, only 30 min sonication time is necessary, and the analytical features (precision, limit of detection, and accuracy) indicated that the proposed analytical method offers a good alternative for the direct determination of copper, iron and manganese in seafood samples.

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