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Analytical Methods

Matrix importance in animal material pre-treatment for metal determination

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Abstract

A relatively cheap and accessible way to analyze difficult samples without loss of analyte was optimized for determination of Cd, Cr, Cu, Hg, Ni, Pb, V and Zn in animal material. Reference materials with suitable matrix were used: Dorm-2 (Dogfish Muscle), Tort-2 (Lobster Hepatopancreas), and SRM 2976 (Mussel Tissue). The analytical procedure included digestion of the sample in Parr reactor bombs combined with a commercial microwave oven and metals analysis by atomic absorption spectrometry. A weak digestion procedure was optimized to prevent metal losses: a two-step digestion with an aqueous solution of HNO_3, each step carried at a microwave power of 340 W during 4 min. With the optimized procedure, metals concentrations in all reference materials matched certified ones (for a 95% level of confidence) with the exception of Cd in Tort-2 and Hg in SRM 2976. The present work reinforces the need to use matrix-matched reference materials and that any digestion protocol needs to be modified to maximize recoveries.

Keywords: Microwave digestion; Metals; Fish; Lobster; Mussel; Atomic absorption spectrometry

1. Introduction

It has been recognized for several years that the concentrations of metals found in coastal areas may have a variety of natural and anthropogenic sources (Dalman, Demirak, & Balci, 2006). The major part of the anthropogenic metal load in water and sediments along rivers, estuaries and bays, in most circumstances comes from mining, intensive aquaculture, municipal wastewaters, untreated effluents, harbour activities and urban and agricultural runoff (Dalman et al., 2006).

Metals tend to accumulate in sediments from where they may be released. The accumulation of metals released by sediments into biota and the biological effects associated with metal contamination are important issues that need to be address. Besides, it is becoming increasingly important to understand metal accumulation within food webs, because once these metals reach human beings they may produce chronic and acute illnesses (França, Vinagre, Caçador, & Cabral, 2005). Invertebrates, like mussels, lobsters and fishes are important players in food webs. Mussels, as filter feeders, are known to accumulate metals and are widely used as sentinel organisms to monitor concentrations of metals in coastal environments (Jeng et al., 2000). Metals can also be accumulated by lobsters or fishes either from water or sediment ingestion or through the food chain (Chale, 2002). It is important, therefore, to determine metal loads in these aquatic organisms through, for instance, monitoring programs, to access their real impact in the food webs. Therefore, the selection of appropriated methodologies to accurately determine metal loads in this type of samples is required.

The analysis of metals in solid samples, including animal material like fish, lobster and mussel tissues, requires a

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preliminary decomposition. The decomposition of the sample is a critical step as it can have important effects on the analytical results (Blust, Van der Linden, Verheyen, & Decleir, 1988). One must assure total decomposition of the sample and also that, during decomposition, no losses of the metals that are to be determined occurs. At present there are a large number of methods that are recommended for the preparation of marine samples. The most commonly method used is the microwave digestion, which has been the method of choice in the last years (*e.g.*, Dalman et al., 2006; Jeng et al., 2000; Karadede & Ünlü, 2000; Pérez Cid, Boia, Pombo, & Rebelo, 2001). The advantages of microwave digestion against the classical methods are the shorter time and less consumption of reagents (Smith & Arsenault, 1996), as well as an easy prevention of losses of metals.

Several commercial microwave digestion devices have been referred in the literature. These commercial devices still imply a considerable investment in any Laboratory, because they are invariably costly. A cheaper option is the use of Parr reactor bombs in combination with conventional domestic microwave ovens. Several studies can be found that used Parr reactor bombs, with satisfactory results, for the analysis of different elements in different biological matrixes, like, for instance, fish muscle (*e.g.*, Alonso, Torres, & Pávon, 1992; Pérez Cid et al., 2001), oyster and animal muscle (*e.g.*, Friel, Skinner, Jackson, & Longerich, 1990), mussel tissues (*e.g.*, Yebra & Enríquez, 1998) and lobster hepatopancreas (*e.g.*, Yebra & Enríquez, 1998; Matusiewicz, Sturgeon, & Berman, 1989). Nevertheless, a variety of domestic microwave ovens with different characteristics can be used, being important to optimize carefully each microwave-Parr reactor bomb system to maximize element recoveries.

The aim of this work was the development of a suitable method for the analysis of eight metals, Cd, Cr, Cu, Hg, Ni, Pb, V and Zn, in animal material, namely in fish, lobster and mussel tissues, using microwave digestion in Parr reactor bombs. Appropriated reference materials were selected to develop, evaluate and validate the analytical procedure. Although, it is well known that there is a need to use matrix-matched reference materials, not all studies follow this indication, either for lack of the appropriate reference material or for the lack of the element of choice in the reference material. For instance, Yebra and Enríquez (1998) used lobster hepatopancreas marine reference material to optimize the methodology for determination of cadmium in mussel samples. Pérez Cid et al. (2001) used bovine liver and hay reference materials to validate the method to determine trace metals in fish samples. Bervoets and Blust (2003) used mussel tissue reference material for the determination of Cd, Cu, Cr, Ni, Pb and Zn levels in fish tissues.

Atomic absorption spectrometry (AAS) is one of the commonly used techniques for the determination of metals and was the technique selected in the present work, either with flame atomization (FAAS) or with electrothermal atomization (ETAAS) or with cold vapour of Hg (CV-AAS).

2. Experimental

2.1. Material and reagents

Pro analysis concentrated HNO_3, from Panreac, was used without further purification. All other reagents used were pro analysis grade or equivalent. For AAS measurements, standard solutions of Cd, Cr, Cu, Hg, Ni, Pb, V and Zn were prepared daily from stock AAS standard solutions (1000 mg L^{-1}), from Fluka, in polyethylene tubes.

To prevent contamination, all material was soaked in 20% (v/v) HNO_3 solution for at least 24 h, rinsed several times with filtered bi-deionised H_2O (conductivity $\leqslant 0.054$ μS cm^{-1} (Millipore System)) and dried in a oven.

2.2. Reference materials

Three reference materials were used: Dorm-2, Dogfish Muscle, certified for the concentrations of Cd, Cr, Cu, Hg, Ni, Pb and Zn; Tort-2, Lobster Hepatopancreas, certified for the concentrations of Cd, Cr, Cu, Hg, Ni, Pb, V and Zn; and SRM 2976, Mussel Tissue, certified for the concentration of Cd, Cu, Hg, Pb and Zn and with reference concentrations of Cr and Ni. Dorm-2 and Tort-2 were obtained from the National Research Council of Canada, whereas SRM 2976 was obtained from the National Institute of Standards and Technology, USA.

The material was used as received. The water content in each material was taken into consideration in the final calculation of the certified concentration of metals per *g* of dry material.

2.3. Microwave digestion

Aliquots of each reference material were digested in closed Teflon vessels at high-pressure with different amounts of HNO_3 and H_2O, using Parr reactor bombs (model 4782) and a conventional domestic microwave oven (Panasonic NE-1037). Only one Parr reactor bomb was place in the microwave oven at each time. For the optimization of the microwave digestion the parameters: mass of sample, volume of HNO_3, addition of different volumes of H_2O, addition of 30% H_2O_2 solution, power of the microwave and time of digestion were tested (see Table 1). Water was added to HNO_3 for the microwave digestion for two reasons: one to have enough volume of solution in the Teflon vessel for the digestion of the sample and another to weaken the reaction of HNO_3 (as discussed below).

Preliminary tests showed a significant lost of sample by volatilization (25% reduction in volume) when Parr reactor bombs were allowed to cool slowly at room temperature. These losses were eliminated when an ice bath was used for cooling.

Final solutions were kept at 4 °C until analysis.

Table 1
Different microwave digestion procedures and respective obtained percentages (%) of recoveries relatively to the certified concentration value of Cr in Dorm-2

Microwave procedure	Cr recovery percentages (%)	
Preliminary procedure: *ca.* 0.4 g sample; 1 mL HNO_3 + 3 mL H_2O; 3 min, 500 W	59.4	Solution clear
Procedure 1:[a] *ca.* 0.4 g sample; 4 mL HNO_3 + 1 mL H_2O; 1 min, 340 W	14.8	Solution clear
Procedure 2: *ca.* 0.4 g sample; 1 mL HNO_3 + 2 mL H_2O_2 + 1 mL H_2O; 1 min, 340 W	17.8	Solution clear
Procedure 3: *ca.* 0.4 g sample; 1 mL HNO_3 + 3 mL H_2O; 3 min, 340 W	24.9	Solution not clear
Procedure 4: *ca.* 0.4 g sample; 1 mL HNO_3 + 3 mL H_2O; 3 min, 340 W run twice	57.8	Solution clear
Procedure 5: *ca.* 0.4 g sample; 0.5 mL HNO_3 + 2.5 mL H_2O; 3 min, 500 W	65.8	Solution clear, vessel not clean
Procedure 6: *ca.* 0.3 g sample; 0.5 mL HNO_3 + 2.5 mL H_2O; 3 min, 500 W cooling solution removed to vessel 0.5 mL HNO_3 + 1 mL H_2O; 3 min, 500 W	65.4	Solution clear, vessel clean
Procedure 7: *ca.* 0.3 g sample; 0.5 mL HNO_3 + 2.5 mL H_2O; 4 min, 340 W cooling 0.5 mL HNO_3 to solution; 4 min, 340 W	70.9	Solution clear
Final procedure: *ca.* 0.3 g sample; 0.75 mL HNO_3 + 2.75 mL H_2O; 4 min, 340 W cooling 0.25 mL HNO_3 to solution; 4 min, 340 W	94.7	Solution clear

[a] Based on the procedure of Pérez Cid et al. (2001).

2.4. Metal determination

Metals contents in each reference material were determined by AAS (SpectrAA 220FS, Varian, with background correction through a deuterium lamp).

ETAAS (GTA 110 unit) was used for determination of Cd, Cr, Cu, Ni, Pb and V. Furnace operating conditions were based on those present in the cookbook for food matrixes, being optimized to obtain the maximum sensitivity and precision with the minimum of interferences (Table 2). External calibration with aqueous matched standards was compared with standard addition quantification. Similar results (for a 95% level of confidence) were obtained and external calibration was selected.

FAAS was used for determination of Cr in Dorm-2 and for Zn in all reference materials. Quantification was carried out using default operating conditions and aqueous matched standards. For Cr a nitrous oxide/acetylene flame was used, whereas for Zn an air/acetylene flame was used.

Table 2
ETAAS furnace operating condition optimized[a] for Cd[b], Cr, Cu, Ni, Pb[b] and V

Step	Temperature (°C)	Ramp (s)	Hold (s)
Drying	150	5.0	0.0
	210	50.0	0.0
	260	10.0	0.0
Pyrolysis	700 (Cd)	15.0 (all metals but V)	3.0 (all metals but V)
	800 (Cu, Ni e Pb)	5.0 (V)	22.0 (V)
	1000 (Cr)		
	1800 (V)		
Atomization	1600 (Cd)	1.0	3.0 (all metals but V)
	1800 (Pb)		4.0 (V)
	2400 (Cu)		
	2500 (Cr)		
	2700 (Ni and V)		
Clean up	2800	3.0	0.0

[a] 20 μl of sample were injected in the graphite tube, with the exception of Pb, for which only 15 μl were used; Argon flow was 3.0 L min^{-1}, except for atomization step for which flow was 0 L min^{-1}.

[b] 5 μl of a 1% $(NH_4)_2HPO_4$ solution was used as a chemical modifier.

CV-AAS was used for Hg determinations. A solution of 25% (m/v) of $SnCl_2$ was used as a reducer. Apparatus conditions were automatically selected. External calibration with standards prepared in 0.01% (m/v) $K_2Cr_2O_7$ solution was used for quantification. Although, $K_2Cr_2O_7$ solution is normally used for organic mercury oxidation, the most common oxidant, $NaBH_4$ was not available at our laboratory and preliminary tests indicated that the methodology was suitable for determination of total Hg.

Blank solutions were prepared following the pre-treatment of each sample.

Three independent replicates of each reference material were prepared and analyzed and, after blank subtraction, mean values, standard deviations and respective confidence intervals were calculated.

Statistically significant differences among samples for 5% level of significance were evaluated through *t*-student tests.

3. Results and discussion

3.1. Preliminary microwave digestion procedure

Although for FAAS the amount of HNO_3 present in solution after digestion of each sample is not problematic, for ETAAS the ideal amount is 5% (v/v) or less, because higher amounts can be considered injurious to the graphite furnace. As a result, solutions to be analysed by ETAAS are sometimes subject to high dilution rates. However, some elements present in mussel, lobster and fish tissues are normally at low levels, and, consequently, dilution of a sample becomes a critical step. It is important, therefore, that each sample is diluted as least as possible for analysis. As a result, attempts were carried out to optimize a microwave digestion that required a reduced amount of HNO_3, so that the obtained solution did not have to be subject to high dilution rates when analysis had to be carried by ETAAS.

At first, the three reference materials were subject to a microwave digestion procedure developed that used only 1 mL of HNO_3 (preliminary procedure, Table 1) and in a first stage only Cr, Ni, Pb and V were analysed using the optimized operating conditions. In general, recovery percentages around 100% relatively to the certified concentration value were obtained for the elements determined in Tort-2 and SRM 2976. However, for Dorm-2 recovery percentages of Cr and Ni were around 50%, whereas that of Pb was around 200%. These results indicated that the preliminary microwave digestion was not suitable for the analysis of metals in fish muscle and a different procedure had to be optimized. For some reason that was unknown in this stage of the work, there were losses of Cr and Ni in the digestion procedure of the reference material Dorm-2. For Pb, the high recovery value obtained in this reference material was probably related with measurement errors associated with its low concentration value, which was near the limit of quantification of the analytical procedure. Because of unsatisfactory results, remaining metals were not analysed in reference materials and optimization of the microwave digestion procedure was carried out.

3.2. Optimization of the final microwave digestion procedure

The optimization of the final microwave digestion procedure was carried only with Dorm-2 because for this reference material the microwave digestion seemed to produce defective results, at least for some metals. As in this reference material Cr had a high concentration level, suitable to be analysed by FAAS, this was the only element and the only analytical technique selected in this stage of optimization. Results obtained with the different microwave digestion procedures used through the optimization of a final microwave digestion procedure in terms of percentages of recovery of Cr relatively to the certified concentration value are reported in Table 1.

Assuming that the microwave digestion was not sufficient to decompose the matrix of Dorm-2, which would be reflected in the low percentages of recovery, two stronger microwave digestion procedures were used (procedures 1 and 2, Table 2) and the concentration of Cr determined. The first procedure was based on that of Pérez Cid et al. (2001) for fish tissues. The second procedure was based on the fact that H_2O_2 has often been used in combination with HNO_3 to oxidize organic matter before analysis of animal material (Smith & Arsenault, 1996). The percentage of recovery of Cr obtained with both procedures was *ca.* 4 times lower than that obtain with the preliminary microwave digestion procedure (Table 1). These results indicated that the observed losses of Cr in this reference material were probably not related with inappropriate destruction of the matrix but with losses by volatilization of Cr. As organic matrices are decomposed significant increases in vessels internal pressure are observed due to the formation of CO_2 and NO_2 (Smith & Arsenault, 1996). In addition, the decomposition of the matrix results in the release of volatile chlorine that contributes to the pressure build up inside the vessel (Smith & Arsenault, 1996). Cr in Dorm-2 was probably lost due to vapour release during pressure build up. This was not observed in the other two reference materials probably because they have lower amount of some of the organic matrices constituents, namely carbohydrates, proteins or most likely lipids, and of chloride ions. Therefore, lower amount of volatile compounds are formed during the microwave digestion with less pressure build up and, therefore, with no vapour release. The formation of volatile compounds was probably also the cause for the losses of Ni observed in the preliminary microwave digestion procedure. For Pb, as measurement errors associated with its low concentration value were high, it did not allow to detect analyte losses. Results indicated that a stronger microwave digestion (higher quantity of oxidant or higher power of the microwave) would implicate higher losses. A stronger microwave digestion would increase the pressure

developed inside the reactor, which can trigger vapour release and analyte losses.

These results points outs to the need of a reference material with similar matrix to that of the sample to be analysed.

The next step in the optimization of a suitable microwave digestion procedure was, then, to weaken the digestion, to reduce pressure build up and to prevent the losses of Cr by vapour release. Therefore, the microwave power was reduced (procedure 3, Table 2). But in these conditions the digestion was not complete and the percentage of recovery of Cr was 2 times lower than that obtained with the preliminary procedure. So, the same procedure was repeated but the microwave program was run twice with a cooling step in between (procedure 4, Table 1). With this procedure the digestion was complete but the recovery of Cr was still low, which indicates that there were still losses of Cr.

In the next step, the proportion of HNO_3:H_2O (v/v) was changed, and the proportions 1:1.5, 1:2, 1:2.5, 1:3, 1:4 and 1:5 were tested. From all of those proportions, the only one that produced a significant increase in the recovery of Cr (of about 10%) was that of 1:5 (procedure 5, Table 1). But although the digestion of the sample was complete with this procedure (the solution was clear), the vessels were not clean, presenting a thin coat in the bottom. To try to eliminate this thin coat the mass of sample was reduced (procedure 6, Table 1). Clear solution and clean vessel were obtained but Cr recovery did not improve.

Another approach was then followed and the digestion was once more weakened by reducing the power of the microwave and increasing the time. But with this procedure the digestion would only be complete, and a clear solution would only be obtained, if a second step was introduced, a step in which more HNO_3 was added (procedure 7, Table 1). With this procedure the recovery of Cr increased another 10% (to *ca.* 70%) although losses of Cr were still observed.

These results indicated that to prevent losses of Cr, the digestion had to be smoother: a low microwave power should be selected, and a higher amount of oxidant (in this case HNO_3) should be added. A new procedure was, then, carried out, in which the proportion HNO_3:H_2O was increased (final procedure, Table 1). In this case the second step, in which another aliquot of HNO_3 was added to the solution, was still necessary to allow a complete digestion of the sample. The percentages of recovery for Cr obtain with this microwave procedure increased significantly (*ca.* 35%) to values around 100%, which indicates that losses of Cr were overcome.

3.3. Final analytical procedure for the determination of Cd, Cr, Cu, Hg, Ni, Pb, V and Zn in fish muscle and mussel tissue

The final microwave digestion procedure was applied to all reference materials.

The metals selected were determined in each reference materials and the limits of detection of the entire analytical procedure (microwave digestion and AAS measurement) were estimated. As observed in Table 3, most concentration values obtained matched the certified ones, with no statistically significant differences for a 95% level of confidence. The exceptions were Cd in Tort-2 and Hg in SRM 2976. These results indicate that the analytical procedure optimized is suitable to be used with different matrixes. The high uncertainty associated with the concentration of Pb in Dorm-2 and the higher concentration value of Hg in SRM 2976 was probably related with the low concentration values that were close to the limit of detection of the entire analytical procedure (Table 3). The higher concentration value of Cd in Tort-2 was probably related with the manipulation of the sample since a high dilution rate of the sample had to be carried out for the analysis.

Results indicate that the entire analytical procedure (microwave digestion and AAS measurement) optimized is suitable for the analysis of Cd, Cr, Cu, Hg, Ni, Pb and Zn in fish, lobster and mussel tissues. As for V, because it was present only in lobster tissue, one can still question

Table 3
Metal concentration values[a] obtained in the three reference materials with the final microwave digestion procedure and respective certified concentration values

Metal	LD_{ap}	Dorm-2		Tort-2		SRM 2976	
		Certified	Obtained	Certified	Obtained	Certified	Obtained
Cd	0.0035	0.043 ± 0.008	0.046 ± 0.013	26.7 ± 0.6	29.58 ± 0.67	0.82 ± 0.16	0.92 ± 0.38
Cr	0.026	34.7 ± 5.5	32.80 ± 0.12	0.77 ± 0.15	0.60 ± 0.14	0.50 ± 0.16[b]	0.411 ± 0.089
Cu	0.033	2.34 ± 0.16	2.15 ± 0.20	106 ± 10	100.8 ± 5.7	4.02 ± 0.33	4.5 ± 1.8
Hg	0.035	4.64 ± 0.26	4.50 ± 0.58	0.27 ± 0.06	0.345 ± 0.034	0.0610 ± 0.0036	0.089 ± 0.017
Ni	0.046	19.4 ± 3.1	16.7 ± 2.4	2.50 ± 0.19	2.38 ± 0.25	0.93 ± 0.12[b]	1.027 ± 0.077
Pb	0.047	0.065 ± 0.007	0.065 ± 0.062	0.35 ± 0.13	0.32 ± 0.15	1.19 ± 0.18	1.29 ± 0.32
V	0.61	n.a.	$<LD_{ap}$	1.64 ± 0.19	1.78 ± 0.77	n.a.	$<LD_{ap}$
Zn	3.3	25.6 ± 2.3	25.6 ± 1.4	180 ± 6	198 ± 20	137 ± 13	162 ± 32

n.a.: not available; $<LD_{ap}$: below detection limit of the analytical procedure.

Limits of detection (LD_{ap}) of the entire analytical procedure (pre-treatment and AAS measurement) are also included. Values in $\mu g/g_{dry\ tissue}$.

[a] Mean ± 95% confidence intervals ($n = 3$).

[b] Reference concentrations.

the suitability of the procedure for the determination of this metal in fish muscle and in mussel tissue.

4. Conclusions

The present work stresses out the need of accurately optimizing the pre-treatment step of solid matrixes and the need of a reference material with similar matrix to that of the sample to be analysed. As observed, although a strong and fast microwave digestion procedure would be suitable for the accurate determination of Cd, Cr, Cu, Hg, Ni, Pb, V and Zn in lobster hepatopancreas and mussel tissue, the procedure was not appropriate for the determination of some of these metals in fish muscle, despite similarities of the matrix. For the determination of the selected metals a softer and slower microwave digestion procedure was essential to prevent losses of metals by volatilization.

References

Alonso, E. V., Torres, A. G., & Pávon, J. M. C. (1992). Determination of nickel in biological materials after microwave dissolution using inductively coupled plasma atomic emission spectrometry with prior extraction into butan-1-ol. *Analyst, 117*, 1157–1160.

Bervoets, L., & Blust, R. (2003). Metal concentrations in water, sediment and gudgeon (Gobio gobio) from a pollution gradient: Relationship with fish condition factor. *Environmental Pollution, 126*, 9–19.

Blust, R., Van der Linden, A., Verheyen, E., & Decleir, W. (1988). Evaluation of microwave heating digestion and graphite furnace atomic absorption spectrometry with continuum source background correction for the determination of iron, copper and cadmium in Brine Shrimp. *Journal Analytical Atomic Spectrometry, 3*, 387–393.

Chale, F. M. M. (2002). Trace metal concentrations in water, sediments and fish tissue from Lake Tanganyika. *Science of Total Environment, 299*, 115–121.

Dalman, Ö., Demirak, A., & Balci, A. (2006). Determination of heavy metals (Cd, Pb) and trace elements (Cu, Zn) in sediments and fish of the Southeastern Aegean Sea (Turkey) by atomic absorption spectrometry. *Food Chemistry, 95*, 157–162.

França, S., Vinagre, C., Caçador, I., & Cabral, H. N. (2005). Heavy metal concentrations in sediment, benthic invertebrates and fish in three salt marsh areas subjected to different pollution loads in the Tagus Estuary (Portugal). *Marine Pollution Bulletin, 50*, 993–1018.

Friel, J. K., Skinner, C. S., Jackson, S. E., & Longerich, H. P. (1990). Analysis of Biological Reference Materials, Prepared by Microwave Dissolution, Using Inductively Coupled Plasma Mass Spectrometry. *Analyst, 115*, 269–273.

Jeng, M. S., Jeng, W. L., Hung, T. C., Yeh, C. Y., Tseng, R. J., Meng, P. J., et al. (2000). Mussel watch: A review of Cu and other metals in various marine organisms in Taiwan, 1991–98. *Environmental Pollution, 110*, 207–215.

Karadede, H., & Ünlü, E. (2000). Concentrations of some heavy metals in water, sediment and fish species from the Atatuurk Dam Lake (Euphrates), Turkey. *Chemosphere, 41*, 1371–1376.

Matusiewicz, H., Sturgeon, R. E., & Berman, S. S. (1989). Trace element analysis of biological material following pressure digestion with nitric acid–hydrogen peroxide and microwave heating. *Journal Analytical Atomic Spectrometry, 4*, 323–327.

Pérez Cid, B., Boia, C., Pombo, L., & Rebelo, E. (2001). Determination of trace metals in fish species of the Ria de Aveiro (Portugal) by electrothermal atomic absorption spectrometry. *Food Chemistry, 75*, 93–100.

Smith, F. E., & Arsenault, E. A. (1996). Microwave-assisted sample preparation in analytical chemistry. *Talanta, 43*, 1207–1268.

Yebra, M. C., & Enríquez, M. F. (1998). Optimized microwave digestion procedure for cadmium analysis of mussel samples. *Analysis, 26*, 261–263.