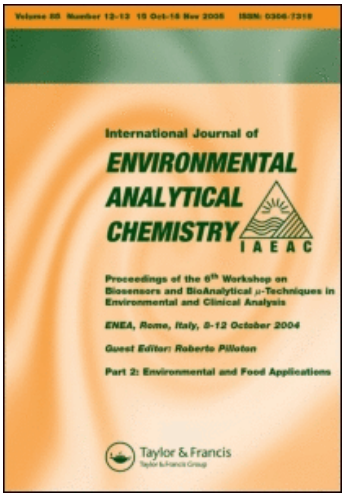

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

A Practical Evaluation of Microwave and Conventional Wet Digestion Techniques for the Determination of Cd, Cu And Zn in Wheat Grain

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Online publication date: 17 September 2010

To cite this Article Adams, Martin L. , Chaudri, Amar M. , Rousseau, Isabelle and Mcgrath, Steve P.(2003) 'A Practical Evaluation of Microwave and Conventional Wet Digestion Techniques for the Determination of Cd, Cu And Zn in Wheat Grain', International Journal of Environmental Analytical Chemistry, 83: 4, 307 — 314
To link to this Article: DOI: 10.1080/0306731031000083988
URL: http://dx.doi.org/10.1080/0306731031000083988

Intern. J. Environ. Anal. Chem., Vol. 83, No. 4, pp. 307–314

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A PRACTICAL EVALUATION OF MICROWAVE AND CONVENTIONAL WET DIGESTION TECHNIQUES FOR THE DETERMINATION OF Cd, Cu AND Zn IN WHEAT GRAIN

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(Received 6 June 2001; In final form 5 October 2001)

The cadmium, copper and zinc concentrations of standard reference materials and wheat samples prepared by microwave digestion and conventional nitric-perchloric acid wet digestion techniques were compared. The two digestion methods both gave acceptably consistent and reliable results for the elements studied. Some practical advantages and disadvantages of both techniques from a laboratory point of view are discussed. Despite one of the major advantages of microwave digestion being the rapidity of digestion, for large numbers of samples the combined restraints of small batch sizes, vessel cleaning time and system cost means analysis by conventional means is often as rapid and less expensive than by microwave. Furthermore analysis by conventional means is capable of achieving results of an equivalent analytical precision and accuracy.

Keywords: Closed-vessel digestion; Microwave dissolution; Plant analysis

INTRODUCTION

The metal content of foodstuffs is an increasingly important aspect of food quality and safety. New food regulations, such as the recently introduced European limits specifying the maximum allowable contaminant levels for several elements in a range of foodstuffs [1] illustrate the continuing attention given to food safety with respect to potentially toxic metals. Apart from human health concerns, the presence of non-essential and potentially toxic metal in agricultural produce can have serious implications for international trade, with quality issues effectively becoming "non-tariff" barriers to trade. It will therefore become increasingly important for both food producers and exporters to be able to demonstrate and certify that their produce complies with the relevant legislative standards for toxic metals. This can only be achieved through laboratories using appropriate methods of sample preparation and analysis.

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ISSN 0306-7319 print: ISSN 1029-0397 online © 2003 Taylor & Francis Ltd
DOI: 10.1080/0306731031000083988

A large number of methods exist for the elemental analysis of plant material [e.g. 2,3]. A range of modern analytical techniques (e.g. graphite furnace atomic absorption spectroscopy (GFAAS), inductively coupled plasma atomic emission spectroscopy (ICP-AES) and inductively coupled plasma mass spectrometry (ICP-MS)) offer high precision, a high degree of automation and can involve simultaneous multi-element measurements over wide calibration ranges. Sample preparation remains a critical area in trace element analysis, and can influence the subsequent analytical data obtained.

The more traditional methods of sample preparation usually involve dry ashing by muffle furnace or wet digestion/oxidation techniques using heating blocks or hot plates. Such methods have been regarded as being both tedious and overly time-consuming [4,5]. In contrast, commercial closed-vessel microwave digestion systems have become increasingly popular for several reasons [15]. These include reduced sample preparation time due to faster sample breakdown and analyte dissolution, smaller amounts of acid required for digestion, constant pressure/temperature control via monitoring of a control vessel, a high degree of digestion automation, and flexibility in terms of sample preparation [6]. Additionally, the use of closed-vessels reduces the risk of sample cross-contamination during digestion runs, and eliminates the loss of volatile elements such as As, Hg and Se during digestion.

However, not all laboratories have, or can afford closed-vessel microwave systems and many still make use of the conventional hot plate or heating block open-tube digestion methods. Therefore it is important to compare both types of sample preparation techniques. This article compares a closed-vessel microwave digestion system with conventional open-tube digestion by heating-block, two common and widely used techniques for sample preparation. A number of previous reports have studied closed-vessel microwave dissolution systems in comparison with other digestion techniques [e.g. 4,7–9]. However, in contrast to previous studies, this work investigates the utility of the two methods with respect to the determination of the potentially toxic elements Cd, Cu and Zn in both plant tissue standard reference materials and in a number of wheat grain samples in which the concentrations of these elements varied widely.

EXPERIMENTAL

Standard Reference Materials

The digestion and analysis of a number of certified standard reference materials (NIST SRM1567a wheat flour, NIST RM8436 durum wheat flour, NBS SRM1572 citrus leaves and NIST SRM1547 peach leaves) were performed to confirm the validity of the analytical procedures, and as a further means of comparing the effectiveness of the two methods of digestion.

Wheat Samples

Wheat samples (*Triticum aestivum* Cv Hereward; $n = 64$) were hand harvested from experimental plots in August 1999 at the site of a long-term sewage sludge field experiment at ADAS Rosemaund, Herefordshire, United Kingdom. Sludges 'naturally' rich in Zn, Cu, Ni or Cr had been applied in 1968 to plots at a rate of 125 tds ha^{-1}, with control non-metal enriched sludge used where necessary to make up quantities and allowing a range of soil metal concentrations to be obtained. The Cr-rich sludge was also

contaminated with Cd. The established experiment consisted of eight metal treatments, together with an untreated soil and an uncontaminated sludge control treatment in a randomised block design with four replicate plots per treatment. Further experimental and site details are described in Chaudri *et al.* [10]. After harvesting, the whole grain was milled into flour ($< 150\,\mu m$) and representative subsamples taken and dried at 80°C for 12 h before analysis.

Digestion and Analytical Procedures

All glassware and microwave vessels were acid-washed and thoroughly rinsed with ultra-pure deionised water before use. Microwave vessels were also regularly cleaned by microwaving capped vessels containing 20 mL of 50% nitric acid solution for 10 min at 175°C.

(a) Closed-vessel Microwave Digestion Protocol

Samples (*ca.* 1 g) of dried and ground plant material were digested in teflon® PFA microwave liners (CEM Corp, Matthews, NC) using 3 mL Primar ultra-pure concentrated nitric acid (70% (w/v)) (Fisher Scientific), 2 mL of Primar 30% (w/v) hydrogen peroxide (Fisher Scientific) and 7 mL ultra-pure (18 MΩ specific resistance) water (ELGA Maxima, High Wycombe, UK). A CEM Mars X model microwave (CEM Corp.) equipped with a 12 sample carousel was used for sample digestion. The in-built CEM system software was used to control the digestion conditions of the microwave, using a control vessel constantly monitored for pressure and temperature control. Details of the microwave heating program are given in Table I. After completion of the heating process the vessels were cooled and samples transferred to 25 mL volumetric flasks (Fisherbrand Class A, Fisher Scientific), before being made up to volume with ultra-pure H_2O. Quality assurance throughout the analytical process was maintained by the routine inclusion of National Institute of Standards and Technology (NIST) standard reference material 1567a wheat flour and reagent blanks in each microwave digest batch of twelve samples. Sample replicates were also regularly analysed every *ca.* 15 samples.

(b) Conventional Open-tube Wet Digestion Protocol

A HNO_3–$HClO_4$ digestion protocol based on that described by Zhao *et al.* [11] was used. A brief description of the method follows. Samples (*ca.* 0.5 g) of dried and ground plant material were weighed into 30 mL graduated borosilicate Pyrex® boiling tubes and 5 mL of mixed acids (85 parts concentrated HNO_3 (70%) and 15 parts $HClO_4$ (70%) Primar, Fisher Scientific) were added. Suitable laboratory precautionary measures should be taken when using $HClO_4$ for digestion purposes. In this digestion protocol, the

TABLE I Closed-vessel microwave digestion program (CEM Mars X)

Stage	*Max power* (W)	*Temperature* (°C)	*Ramp* (min)	*Hold* (min)	*Max pressure* (psi)
1	1200	115	8	1	450
2	1200	175	12	10	450

TABLE II Open-tube heating block digestion program

Stage	*Ramp rate* (°C h^{-1})	*Dwell temp.* (°C)	*Dwell time* (h)
1	60	60	3
2	120	100	1
3	120	120	1
4	50	195	2.5

possibility of hazardous reactions occurring are minimized through the use of both small volumes of the reagent itself and small sample sizes. Tubes were mixed by whirlimixer, and left to stand for 2 h at room temperature. Digestion was performed using a 54 sample capacity Carbolite heating block connected to a microprocessor-controlled Eurotherm 818 controller/programmer (Carbolite, Derbyshire, UK). Details of the heating block program are given in Table II. After digestion to dryness (or near dryness) and cooling, 5 cm^3 of HNO_3 (25% (v/v)) were added to each tube, whirlimixed and re-warmed at 80°C for 30 min, and for a subsequent further 30 min after adding ultra-pure water to near the 20 cm^3 mark. After cooling, the solution was made up to 20 cm^3 in each tube. Ten percent of the samples were analysed in duplicate. The reliability of the digestion and analytical procedure was tested by the routine inclusion of two blanks and two NIST standard wheat flour (SRM 1567a) samples with every batch of 50 sample digests.

Cadmium was determined in the digest solutions using a Perkin-Elmer 4100ZL graphite furnace atomic absorption spectrophotometer (Perkin-Elmer, Norwalk, CT) with Zeeman background correction. ICP-AES-Accuris was used to measure Cu and Zn in the digest samples. Plant concentrations are reported on a dry-weight basis. Statistical analyses were performed using Genstat 5 [12].

RESULTS AND DISCUSSION

Standard Reference Materials

The Cd, Cu and Zn elemental analysis data summarised in Table III show that, within experimental error, both the microwave and open-tube digestion methods gave reliable and reproducible results when compared with the certified values of the standard reference materials. A slight reduction in the recovery of Zn was apparent for one sample (NIST peach leaf, Table III), with the difference falling outside the confidence interval limits. Whether this was due to an incomplete digestion, or an analytical/matrix problem could not be determined. There was good agreement between the certified values and the determined concentrations for the remaining elements and digestion methods, with no sizeable or consistent differences between the two digestion methods. These results confirm the appropriateness of the selected experimental conditions and suitability of the digestion protocols for this type of analysis of plant material.

Wheat Samples

The 64 wheat grain samples harvested from the long-term sewage sludge field experiment contained a wide range of Cd, Cu and Zn concentrations (Figure 1). The uptake of such varying amounts of metal by the wheat reflects the underlying

TABLE III Mean concentrations ($\mu g\,g^{-1}$) determined in 6 replicate digests of each standard reference material analysed using either microwave or open-tube digestion methods. Uncertainties are expressed as 95% confidence intervals

Sample	*Cadmium*			*Copper*			*Zinc*		
	Certified	*Microwave*	*Open-tube*	*Certified*	*Microwave*	*Open-tube*	*Certified*	*Microwave*	*Open-tube*
NIST SRM1567a Wheat flour	0.026 ±0.002	0.026 ±0.001	0.026 ±0.003	2.1 ±0.2	2.2 ±0.05	2.3 ±0.06	11.6 ±0.4	12.0 ±0.27	10.7 ±0.55
NIST SRM1547 Peach leaves	0.026 ±0.003	0.027 ±0.001	0.032 ±0.002	3.7 ±0.4	3.6 ±0.02	3.6 ±0.11	17.9 ±0.4	17.0 ±0.17	15.3 ±0.92
NBS SRM1572 Citrus leaves	0.03 ±0.01	0.037 ±0.004	0.039 ±0.003	16.5 ±1.0	18.0 ±2.65	15.4 ±0.36	29 ±2	27.8 ±0.95	25.8 ±1.00
NIST RM8436 Durum wheat flour	0.11 ±0.05	0.118 ±0.005	0.095 ±0.009	4.30 ±0.69	4.2 ±0.01	4.4 ±0.05	22.2 ±1.7	21.5 ±0.25	20.3 ±0.69

differences in metal soil concentrations and bioavailabilities between plots at the experimental site. Figure 1 also illustrates the good agreement apparent between the analytical results from the microwave digest solutions and those obtained from the open-tube method. Results for each of the elements determined by the two digestion methods were significantly correlated ($P < 0.001$), with fitted lines for the Cd, Cu and Zn comparisons having r^2 values of 0.96, 0.89 and 0.98 respectively, and slopes close to the theoretical value of 1.

The good agreement between the two methods of sample preparation for both the standard reference materials and the wheat samples over the wide range of concentrations observed, indicates the suitability of both types of digestion method for the analysis of samples of this nature. As in this study, a comparative study of five digestion methods [9] found close agreement between the elemental concentrations of various food samples prepared using HNO_3–H_2O_2 microwave digestion and HNO_3–$HClO_4$ wet digestion techniques. It was concluded that, except for the elements Al and B, the HNO_3–$HClO_4$ wet digestion technique was the simplest and most effective procedure. Although concentrated $HClO_4$ can have a deleterious effect on GF tube life, in our study these effects, and hence also any associated matrix effects, were much reduced because of the digestion protocol used. Specifically, of the small volume of $HClO_4$ initially used in the digestion (0.75 mL), a substantial amount is consumed during the digestion itself. Any residual amount that may remain after the heating process is subsequently diluted as the digest solution is made to volume with HNO_3 and H_2O. The concentration of $HClO_4$ in the final solution is therefore $< 1\%$. Perhaps the best indication that no adverse effects occurred from the use of $HClO_4$ was given by the excellent agreement of digested samples with the certified values, with no correction for matrix effects necessary (Table III). Additionally, no reduction in furnace tube lifetime was observed during the analysis of these samples.

The introduction of microwave technology has resulted in a large number of protocols that allow for the rapid digestion of samples [5]. While the increased rapidity of digestion is certainly advantageous for smaller sample numbers, the speed of the microwave digestion procedure becomes less important as the number of samples increases [4]. For larger sample numbers, significant throughput restraints are imposed by the ability to digest only small numbers of samples in one digestion batch, the

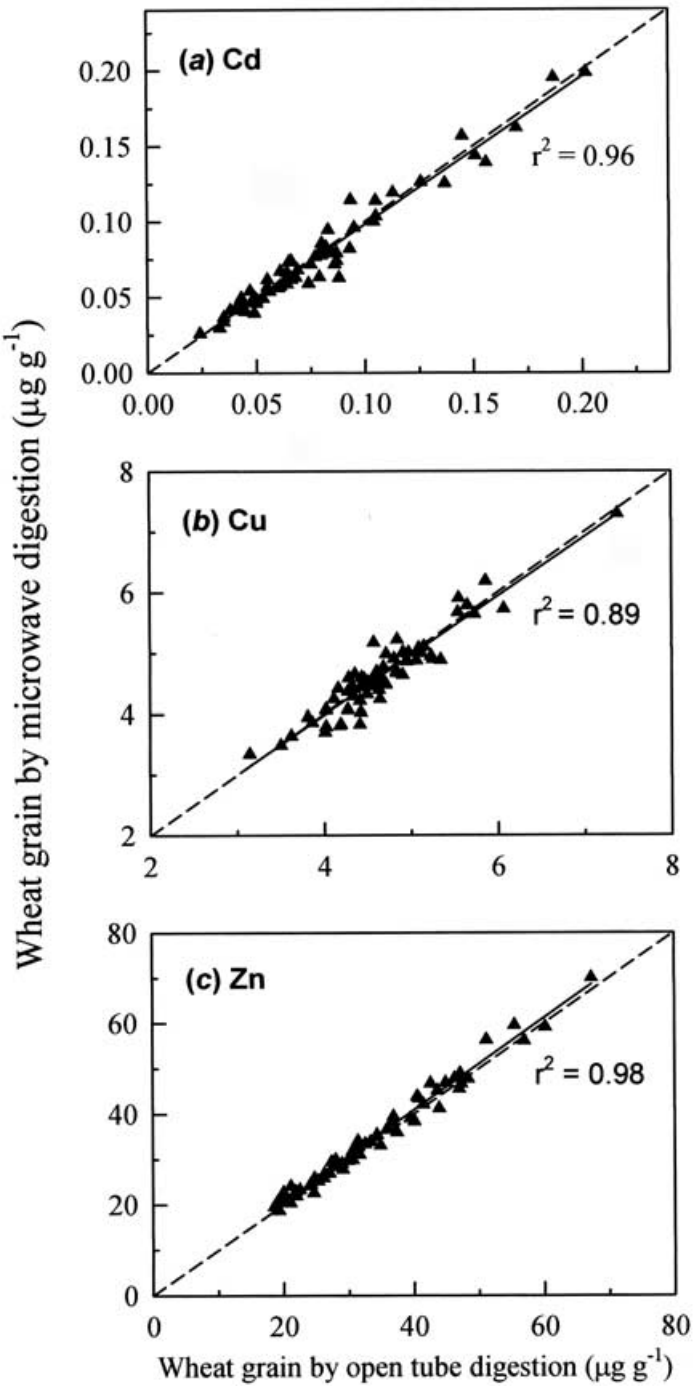

FIGURE 1 Comparison of microwave and conventional open tube digestion techniques for determination of (a) Cd; (b) Cu; and (c) Zn concentrations in wheat grain using samples from the ADAS-Rosemaund UK experimental site. The solid line indicates the fitted regression between the two data sets for each respective plot, whilst the dashed line shows a 1 : 1 relationship.

cooling time required before vessel venting can occur and the time required to adequately clean (either acid-wash or acid-heating in microwave) vessels to the standard of cleanliness required for determining trace metal concentrations. The recent development and commercial availability of continuous-flow microwave dissolution techniques [5,13,14] has, however, helped improve the sample throughput of microwave digestion methods.

Conventional batch microwave systems can generally contain up to 12 sample vessels in one batch, but a number will be occupied by reagent blanks, standard materials and/or sample repeats for QC purposes. The cost of purchasing numerous additional sets of spare vessels will be prohibitive for most laboratories, and for large batch sizes a limiting factor for throughput is often the time required to acid-wash the vessels in preparation for subsequent batches. In contrast, standard heating blocks of the type used in this work can accommodate 50 or more digestion tubes, and although the digestion process itself is of longer duration, the use of an automated heating block controller allows digestions to be run overnight. The cost of borosilicate Pyrex® digestion tubes is also considerably lower than that of microwave vessels, and therefore the purchase of additional complete sets of vessels to reduce the throughput bottleneck imposed by cleaning is a more practical proposition. A further advantage of the HNO_3–$HClO_4$ wet digestion is that no solution transfers are required, so that analytical errors due to solution transfer are minimised [9].

A comparison of the costs involved for the two digestion methods used in this study reveals a similar reagent cost for the two techniques. However, microwave vessels require a single-use rupture membrane for safety purposes, which increases the cost per sample of a microwave digest to around 2.5 times the cost of a sample digested by the conventional open-tube method. The initial outlay required for a microwave digestion is also significant, and may be more than 5 times that required to purchase a heating block similar to that used in this work. Microwave vessels also have a finite lifetime and need regular replacement, as each vessel can only be used for around 100 digestions [15]. Nevertheless, the significant advantages of microwave digestion technology such as its suitability for analysis of a wide variety of sample types [5,15], and its ability to eliminate gaseous losses from samples containing volatile elements [5,9] may well outweigh these limitations, as well as the significant initial cost of the microwave unit itself.

CONCLUSIONS

The two digestion methods described both offer effective protocols for the preparation of plant material samples prior to analysis of potentially toxic metals. Good agreement was obtained between the analytical results of standard reference materials and experimental field samples obtained using the two contrasting procedures, and both methods therefore appear suitable for the digestion of plant matter prior to elemental analysis. In laboratories where the trace metal content of large numbers of samples is required, conventional open-tube digestion methods may be cheaper and equally as effective as digestion by microwave. However, closed-vessel microwave digestion techniques remain indispensable should the preparation and analysis of samples containing volatile elements be required.

Acknowledgements

The authors thank ADAS staff for their assistance in providing access to samples from the Rosemaund experimental site. This work was partially funded by the UK Home-Grown Cereals Authority. Rothamsted Research receives grant-aided support from the UK Biotechnology and Biological Sciences Research Council.

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