

SHORT COMMUNICATION

VALIDATION OF MICROWAVE DIGESTION METHOD FOR DETERMINATION OF TRACE METALS IN MUSHROOMS

ANTUN KUČAK¹ AND MAJA BLANUŠA²

Mining and Chemistry School, Varaždin¹, Institute for Medical Research and Occupational Health, Zagreb², Croatia

Received 12 November 1998

A microwave digestion method for mushrooms, developed in the study, allows fast preparation of samples and reduces the contamination risk in the process of determining trace metals. Concentrations of six trace elements, Fe, Mn, Cu, Zn, Pb, and Cd were measured in 50 samples of different species of edible mushrooms (fam. *Boletaceae*) using atomic absorption spectrometry after microwave and dry ashing procedure. The methods were validated through certified Standard Reference Material SRM 1577b (Bovine Liver) which was treated and analysed using the same procedures as for the mushrooms. The samples were either digested with concentrated HNO₃ in closed Teflon PFA vessels in a microwave oven, or ashed in quartz crucibles at 450 °C. The respective recoveries of Fe, Mn, Zn, Cu, Pb, and Cd obtained by measuring SRM were 112, 107, 104, 115, 111 and 95% after microwave digestion procedure and 86, 101, 109, 111, 98, and 110% after dry ashing procedure. The correlation between concentrations obtained by the two different methods of sample preparation was high for all metals.

Key words:
cadmium, copper, dry ashing, iron, lead, manganese, plant tissue, zinc

Sample preparation is a critical step in trace metal analysis by atomic absorption spectrometry. A classical digestion method requires hours or days to ensure complete destruction of the sample (1).

Microwave heating is relatively new but a widely used laboratory method for heating liquids. Mineral acids absorb microwave energy and heat very rapidly. The

greatest reduction in digestion time comes from combining the speed of microwave heating with the elevated temperature and pressure achieved in sealed Teflon PFA vessels. Preparation procedures, which require ashing or fusing the sample prior to acid digestion, can now be replaced by direct digestion of the sample in acids using microwave heating under pressure. It is often possible to accomplish digestions that normally require the use of perchloric acid by using nitric acid alone in a closed vessel system (2, 3). Standard reference materials (SRMs) should provide reliable means to establish loss or contamination of trace elements during sample preparation. However, it has been reported that a precipitate was observed in some SRMs after microwave digestion, which led to suspicion that the organic matrix was only partially destroyed (4). In order to prove the reliability of the method, the SRM should be of the same or similar biological composition as the sample to be analysed.

Mushroom is a very specific sample for destruction. It contains plant oils and chitin in the cell membrane which is difficult to destroy. This study sought to compare and verify two methods of mushroom digestion for the analysis of several trace metals. The verification was based on certified SRM Bovine Liver (a certified mushroom SRM was not available) and trace elements were analysed using the atomic absorption spectrometry (AAS).

MATERIALS AND METHODS

Reagents and equipment

Working standards for measurements of Fe, Mn, Zn, Cu, Pb, and Cd were prepared from Spectrosol (BDH, Great Britain) solutions of 1 mg/L each. Certified Standard Reference Material SRM 1577b (Bovine Liver) from the National Institute of Standards and Technology (NIST, USA) was used to verify the methods. Nitric acid (65%, p.a., Kemika, Zagreb) used for the preparation of standards and sample solutions was distilled in the laboratory. Deionised water was purified by ion exchange (Labconco, USA) to 0.06 $\mu\text{S}/\text{cm}$ conductivity.

Dry ashing was carried out in Gallencamp muffle furnace (Great Britain). A closed microwave system, CEM MDS-2000 (USA), was used for wet digestion in the study. Flame atomic absorption measurements were carried out on Varian® model AA 375 (Australia). Flame AAS was used to determine Fe, Mn, Zn, Cu, Pb, and Cd in an acetylene-air flame. Only Pb in Bovine Liver was analysed by electrothermal AAS on Varian® model AA 300. Table 1 shows the conditions for determination of the trace metals. Both instrumental methods were carried out with deuterium background correction.

Table 1 *Experimental conditions for trace metal determinations by AAS*

| | Fe | Mn | Zn | Cu | Pb | Cd |
|-----------------|-------|-------|-------|-------|-------|-------|
| Wavelength (nm) | 248.3 | 279.5 | 213.9 | 324.7 | 217.0 | 228.8 |
| Slit width (mm) | 0.2 | 0.2 | 0.5 | 0.5 | 1.0 | 0.5 |

Sample preparation

Fifty samples of mushrooms were collected from ten sites in the Varaždin county in Croatia. All edible species belonged to fam. *Boletaceae*. Damaged, old, or dirty specimens were removed. The mushrooms were cleaned from dirt, chopped up with a plastic knife, air-dried, and then frozen. Each mushroom sample was analysed in duplicate. Lyophilised SRM Bovine Liver samples were dried at 105 °C before dry ashing or microwave digestion.

Dry ashing

Samples of mushrooms and SRM Bovine Liver, about 0.5 g each, were put in quartz crucibles. Samples were dry ashed overnight in a furnace gradually increasing the temperature up to 450 °C. Empty crucibles in the furnace were used as blank samples. After ashing, the crucibles were left in the furnace to cool until the next day. The ash was dissolved in 1.5 ml of concentrated nitric acid by heating on a hot plate. After dissolution, deionised water was added to the mixture to reach the mass of 10 g.

Microwave digestion

At first, washing and preparation of advanced composite vessels for microwave digestion followed the procedure described in the instructions manual. The instructions, however, proved unsatisfactory, as trace metals in SRM Bovine Liver yielded excessive values. The washing procedure was modified the following way. Teflon PFA vessels with covers and vent fittings were soaked in nitric acid 10% v/v overnight. The vessels and the covers were rinsed with deionised water, soaked in a 3% ethylenediaminetetraacetic acid (EDTA) for 60 minutes, then rinsed again with deionised water, and eventually dried with cell-tissue.

About 0.5 g of dry sample of mushrooms or SRM Bovine Liver was weighed and digested with 1.5 ml of concentrated nitric acid. After one hour of microwave digestion, 3.5 ml of deionised water was added into each vessel. Eleven vessels were filled with samples and the twelfth was used as a blank (5). After many attempts which involved changing of power and pressure, we established the most satisfactory programme for mushroom digestion (Table 2). The digestion took about 70 minutes and cooling another 30 minutes. Vessels were opened in a fume cupboard and filled with

Table 2 The digestion program for mushrooms using microwave oven

| Step | Power (%) | Pressure (psi*) | Time 1** (min) | Time 2*** (min) |
|------|-----------|-----------------|----------------|-----------------|
| 1 | 50 | 20 | 15 | 7 |
| 2 | 65 | 45 | 10 | 7 |
| 3 | 80 | 80 | 10 | 7 |
| 4 | 95 | 110 | 10 | 7 |

* 1 psi = 6 895 Pa = 69 mbar

** time to reach preset pressure

*** time to hold preset pressure

deionised water to reach the sample mass of 10 g. Small quantities of Fe, Zn, and Cd found in blank samples were subtracted from the sample values.

Statistical evaluation

All results are expressed as mg/kg of dry sample. Pearson's correlation test was applied to correlate the two digestion methods for mushrooms. Data were processed for statistics using Stat for Windows[®] and Microsoft[®] Excel.

RESULTS AND DISCUSSION

Table 3 shows the results of Fe, Mn, Zn, Cu, Pb, and Cd analysis obtained by measuring certified SRM Bovine Liver. The respective recoveries of Fe, Mn, Zn, Cu, Pb, and Cd were 112, 107, 104, 115, 111, and 95% after microwave digestion and 86,

Table 3 Certified and obtained concentrations (mg/kg of dry weight) of trace elements in SRM 1577b Bovine Liver using various methods of sample preparation

| | Fe | Mn | Zn | Cu | Pb | Cd |
|----------------------|----------|------------|----------|---------|---------------|---------------|
| Ref. value | 184 ± 15 | 10.5 ± 1.7 | 127 ± 16 | 160 ± 8 | 0.129 ± 0.004 | 0.500 ± 0.03 |
| Dry ashing* | 159 ± 19 | 10.6 ± 0.1 | 138 ± 4 | 178 ± 2 | 0.127 ± 0.002 | 0.550 ± 0.016 |
| Recovery (%) | 86 | 101 | 109 | 111 | 98 | 110 |
| Microwave digestion* | 206 ± 4 | 11.2 ± 0.2 | 132 ± 2 | 184 ± 5 | 0.143 ± 0.018 | 0.477 ± 0.017 |
| Recovery (%) | 112 | 107 | 104 | 115 | 111 | 95 |

*Results are presented as arithmetic means ± standard deviation of four replicates

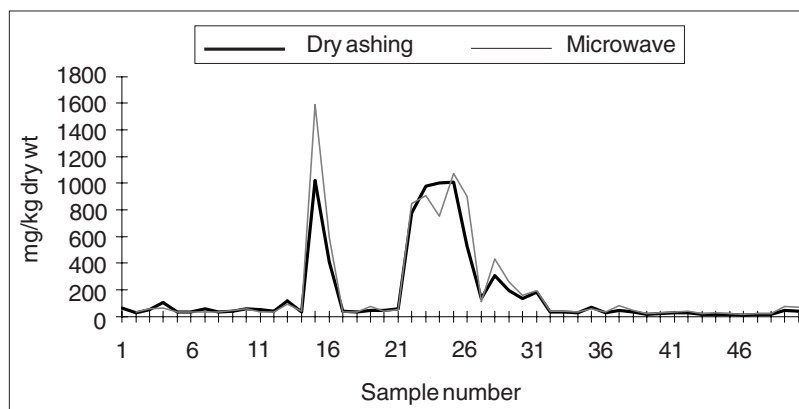


Figure 1 Concentrations of Fe obtained by dry ashing and microwave digestion of 50 mushroom samples. Coefficient of correlation dry ashing/microwave digestion, $r=0.953$

101, 109, 111, 98, and 110% after dry ashing. Figures 1-5 show concentrations of trace elements obtained from fifty mushroom samples applying the two decomposition methods. The correlation between the concentrations obtained through the two methods was rather high. Coefficients of correlation (r) were high and ranged from 0.748 for Cu to 0.953 for Fe. Dry ashing of SRM Bovine Liver gave lower and microwave digestion higher results than expected for iron. Nevertheless, the high and significant coefficient of correlation for this element obtained in mushrooms shows good

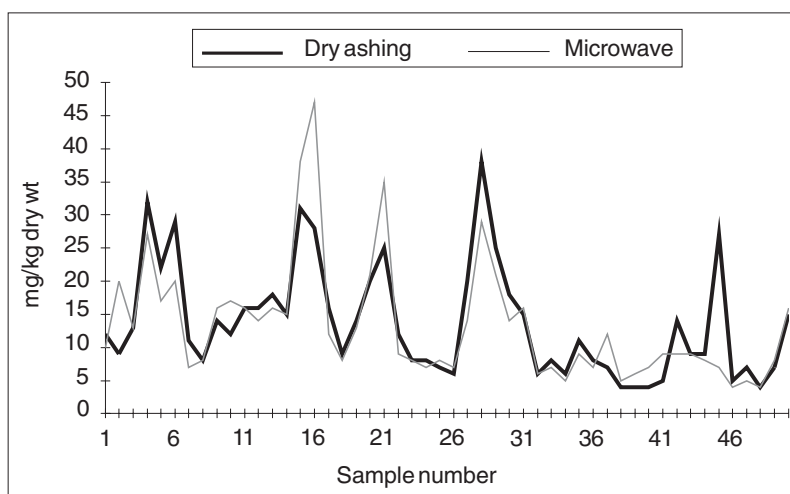


Figure 2 Concentrations of Mn obtained by dry ashing and microwave digestion of 50 mushroom samples. Coefficient of correlation dry ashing/microwave digestion, $r=0.802$

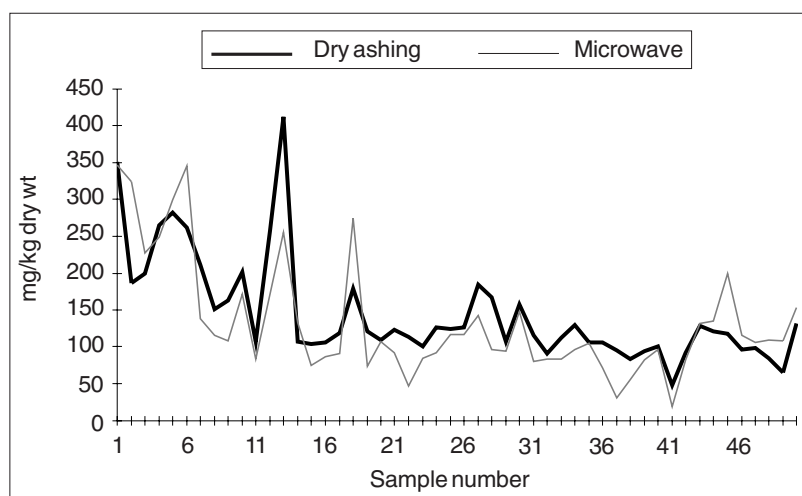


Figure 3 Concentrations of Zn obtained by dry ashing and microwave digestion of 50 mushroom samples. Coefficient of correlation dry ashing/microwave digestion, $r=0.764$

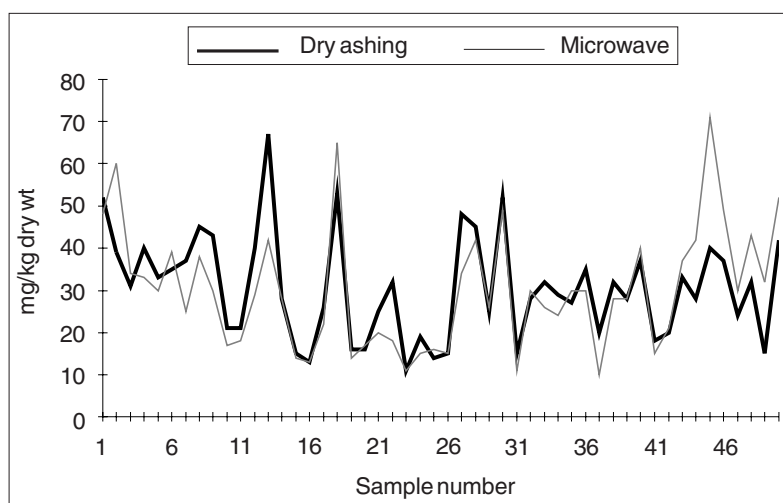


Figure 4 Concentrations of Cu obtained by dry ashing and microwave digestion of 50 mushroom samples. Coefficient of correlation dry ashing/microwave digestion, $r=0.748$

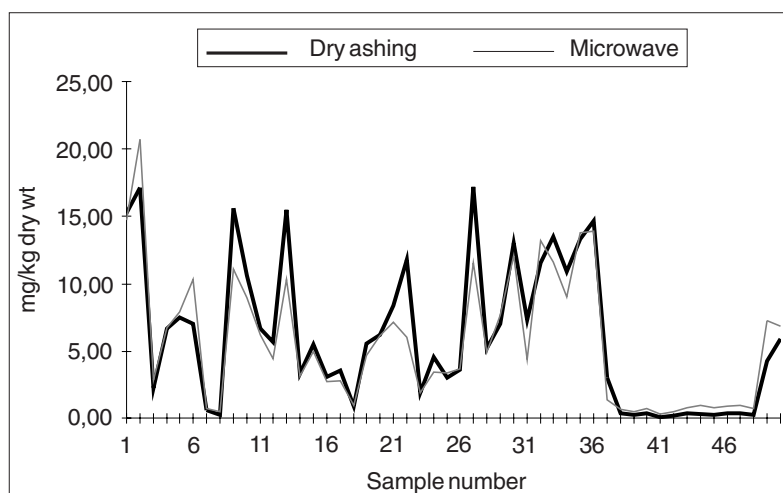


Figure 5 Concentrations of Cd obtained by dry ashing and microwave digestion of 50 mushroom samples. Coefficient of correlation dry ashing/microwave digestion, $r=0.937$

agreement between the two methods. This indicates that SRM Bovine Liver is not an adequate reference material for verification of iron analysis in mushrooms. Data for lead in mushrooms are missing, because its concentration was lower than was the detection limit of flame AAS analysis (<0.8 mg/kg). The alternative method, electrothermal AAS of lead could not be done because no solution or mushroom sample was left over after the flame AAS measurement of five elements. The detection limits for other elements Fe, Mn, Zn, Cu, and Cd by flame AAS were 0.76, 0.26, 0.18, 0.24, and 0.06 mg/kg, respectively.

Conventional dry ashing of plant material samples slows down the process tremendously. Another problem with conventional digestion includes formation of oxides or elemental occlusion to siliceous particles in the sample (6). During dry ashing, iron forms oxides which are difficult to dissolve. This might be the cause of too low recovery of iron after dry ashing of SRM Bovine Liver.

To conclude, microwave digestion seems to be a reliable and rapid method for decomposition of mushroom samples for flame AAS measurements of five elements: iron, manganese, zinc, copper, and cadmium.

Acknowledgements The authors wish to thank Ms. Đurđa Breški and Ms. Marija Ciganović for valuable technical assistance.

REFERENCES

1. *Stripp RA, Bogen DC*. The rapid decomposition of biological materials by using a microwave acid digestion bomb. *J Anal Toxicol* 1989;13:57-9.
2. *Lippo H, Sarkela A*. Microwave dissolution method for the determination of heavy metals in biomonitors using GFAAS and flame AAS. *At Spectrosc* 1995;16:154-7.
3. *Demura R, Tsukada S, Yamamoto I*. Rapid determination of trace metals in foods by using the microwave oven-digestion method. II. Determination of zinc, copper, manganese, lead and cadmium. *Eisei Kagaku* 1985;6:405-9.
4. *Quevauviller P, Imbert J-L, Olle M*. Evaluation of the use of microwave oven systems for the digestion of environmental samples. *Mikrochim Acta* 1993;12:147-54.
5. *Matusiewicz H, Sturgeon RE, Berman SS*. Trace element analysis of biological material following pressure digestion with nitric acid-hydrogen peroxide and microwave heating. *J Anal At Spectrom* 1989;4:323-7.
6. *Rechcigl JE, Payne GG*. Comparison of a microwave digestion system to other digestion methods for plant tissue analysis. *Commun Soil Sci Plant Anal* 1990;21:2209-18.

*Sažetak***VREDNOVANJE METODE MIKROVALNE RAZGRADNJE GLJIVA PRI ODREĐIVANJU METALA U TRAGOVIMA**

Cilj rada bio je razviti pogodnu metodu razaranja organskih uzoraka s pomoću mikrovalova i usporediti rezultate s klasičnom metodom suhog spaljivanja u mufolnoj peći. Nakon razaranja uzoraka izmjerene su koncentracije Fe, Mn, Cu, Zn, Pb i Cd u gljivama metodom atomske apsorpcijske spektrometrije (AAS) plamena. Djelotvornost obiju metoda spaljivanja provjerena je usporedbom rezultata iste metode primijenjene na standardni referentni materijal goveđe jetre. Pedeset uzoraka gljiva različitih rodova vrganjevki sakupljeno je na deset lokacija Varaždinske županije. Provedena je korelacijska analiza između oba postupka spaljivanja za svaki metal.

Suho spaljivanje gljiva izvedeno je u kvarcnim lončićima u peći za spaljivanje pri 450 °C preko noći. Nakon spaljivanja pepeo je otopljen u dušičnoj kiselini. Mikrovalno spaljivanje gljiva provedeno je s pomoću dušične kiseline u zatvorenim teflonskim posudama u mikrovalnoj pećnici. Kao standardni referentni materijal upotrijebljen je SRM 1577b – goveđa jetra. Svi metali određivani su metodom atomske apsorpcijske spektrometrije (AAS) plamena, osim olova koje je određeno elektrotermalnom AAS.

Korelacija između koncentracija dobivenih s pomoću različitih metoda razaranja uzoraka gljiva statistički je značajna za sve određivane metale (koeficijent korelacije $r=0,748-0,953$). Rezultati pokazuju da je mikrovalno razaranje pouzdano i brza metoda za razaranje organskih uzoraka.

Ključne riječi:

bakar, biljno tkivo, cink, kadmij, mangan, olovo, suho spaljivanje, željezo

Requests for reprints:

Antun Kućak, M.Sc.
Mining and Chemistry School
Hallerova aleja 3, HR-42000 Varaždin, Croatia
E-mail: akucak@usa.net