

Comparison of Digestion Methods for Determination of Trace and Minor Metals in Plant Samples

I. Lavilla, A. V. Filgueiras, and C. Bendicho*

Departamento de Química Analítica y Alimentaria, Facultad de Ciencias (Química), Universidad de Vigo, As Lagoas-Marcosende s/n, 36200 Vigo, Spain

In this paper, three dissolution methods using pressure digestion vessels (low-, medium-, and high-pressure vessels) for the determination of metals in plant samples are described. The Plackett–Burman saturated factorial design was used to identify the significant factors influencing wet ashing and to select optimized dissolution conditions. The three methods were statistically compared (one-way ANOVA) on the same sample; no significant differences were obtained. In all cases the relative standard deviation values were <3%. The digestion method based on the use of low-pressure vessels and a microwave oven was validated against CRM GBW07605 tea leaves. This method was applied to the determination of Cu, Zn, Mn, Fe, Mg, and Ca in 22 different medicinal, aromatic, and seasoning plants by flame-atomic absorption spectrometry. The concentration intervals of metal in the plants analyzed were the following: Cu, 4 (*Allium sativum*)–35 (*Thea sinensis*) $\mu\text{g g}^{-1}$; Zn, 7 (*Piper nigrum*)–90 (*Betula alba*) $\mu\text{g g}^{-1}$; Mn, 9 (*Allium sativum*)–939 (*Caryophyllus aromaticus*) $\mu\text{g g}^{-1}$; Fe, 33 (*Allium sativum*)–2486 (*Anethum graveolens*) $\mu\text{g g}^{-1}$; Mg, 495 (*Allium sativum*)–7458 (*Ocimum basilicum*) $\mu\text{g g}^{-1}$; Ca, 386 (*Allium sativum*)–21500 (*Ocimum basilicum*) $\mu\text{g g}^{-1}$.

Keywords: Screening factorial design; plant samples; closed-vessel digestion; microwave; trace and minor metals; atomic absorption spectrometry

INTRODUCTION

The determination of the metal content in medicinal, seasoning, and aromatic plants has received increased attention during recent years because some of these metals are related to human health (Majid et al., 1995). The analytical control of metals in plants and plant-derived products is part of quality control and has for many years been a fundamental feature of ecological and chemical research activity (Chizzola and Franz, 1996).

Metal determination in this matrix has traditionally been carried out using wet or dry ashing methods (Mohd et al., 1992) as sample pretreatment. Of the two methods so far, wet ashing seems to have gained the greatest acceptance among workers interested in the analysis of plant samples for metal determination (Kojima et al., 1988).

Wet decomposition can be performed with a concentrated acid or mixture of acids in open or closed systems (i.e., pressurized), by the use of conventional or microwave heating (Matejovic and Durackova, 1994). Digestion processes in open systems usually require constant operator attention and are prone to systematic errors such as contamination and loss of volatile elements (Abu-Samra et al., 1975). These problems can be minimized if wet ashing is carried out in closed vessels.

In this work several digestion methods carried out in closed vessels were attempted: (i) low-pressure digestion vessel (i.e., 120 psi); (ii) medium-pressure digestion vessel (i.e., 1200 psi); and (iii) high-pressure digestion vessel (i.e., 3000 psi). Microwave-assisted digestion was employed in methods i and ii, whereas conventional

heating (i.e., sand bath) was employed in method iii. In the literature there are multiple combinations of acid types (Smith and Arsenault, 1996; Carlosena et al., 1994), nitric, hydrochloric, and hydrofluoric acids and their mixtures being the more widely employed along with closed digestion vessels. Nitric acid is usually added due to its oxidant properties; hydrochloric acid is used less frequently, and hydrofluoric acid is added when a siliceous matrix is present. Several workers recommended adding hydrogen peroxide after oxidation with nitric acid.

In every case studied, the digestion conditions must be established for each individual analytical problem: sample type, metals to be determined, and digestion system used (Mingorance et al., 1993). Factorial design was used in this work for optimization of variables that influence the digestion process (e.g., acid volumes, acid mixture, digestion time, and microwave power). Application of a complete factorial design requires a great many experiments. The Plackett–Burman fractional factorial design was chosen in this work because it allows the main effects of variables to be known with relatively few experiments (Blanco et al., 1994; Lavilla et al., 1998).

The goal of this work was to develop digestion methods that met the following conditions: (i) applicability to a large variety of botanical samples; (ii) validity for a large number of elements using the same digestion conditions; (iii) minimum acid volume being used for digestion; and (iv) robustness of the digestion methods.

EXPERIMENTAL PROCEDURES

Instrumentation. *Vibrational Agate Ball Mill.* An MM 2000 Retsch (Haan, Germany) agate ball mill was used for grinding the plant samples.

* Author to whom correspondence should be addressed (fax +34-986-812382; e-mail bendicho@uvigo.es).

Table 1. Instrumental Parameters Employed in the Atomic Absorption Spectrometer for Determination of Cu, Zn, Mn, Fe, Mg, and Ca in Plants

element	wavelength (nm)	lamp intensity (mA)	slit width (nm)	working range (mg L ⁻¹)
Cu	324.8	15	0.7	0–5
Zn	213.9	15	0.7	0–1
Mn	279.5	20	0.2	0–2
Fe	248.3	30	0.2	0–5
Mg	285.2	4	0.7	0–0.5
Ca	422.7	4	0.7	0–5

Microwave Ovens. Two types of microwave oven, that is, MDS-2000 CEM (Matthews, NC) microwave sample preparation system and 9245 Samsung (Korea) microwave oven, were used. The CEM system was equipped with a pressure monitoring option and allowed to operate at a power of up to 630 ± 50 W (100% full power) programmable in 1% increments. A computer was implemented, which was programmable for 30 multistep programs consisting of up to five stages each. The Samsung oven could operate up to a power of 1000 W (100% full power) in 10% increments.

Digestion Vessels. The acid digestions of plants were carried out in closed PTFE vessels. Three types of reactors were employed: (i) a 120 mL (CEM) low-pressure reactor consisting of vessel body, cap, relief valve, and vent nut; (ii) a 45 mL Parr (Moline, IL) medium-pressure reactor consisting of a bomb body and screw cap; and (iii) a 150 mL high-pressure reactor consisting of a bomb body and screw cap made of steel.

Atomic Absorption Spectrophotometer. A Perkin-Elmer Model 2380 (Norwalk, CT) equipped with a 10 cm burner head was used for metal determination. Perkin-Elmer hollow cathode lamps of Zn, Cu, Fe, and Mn and a Cathodeon hollow cathode lamp of Ca plus Mg were used as radiation sources. The elements were measured under the optimum operating conditions with an air–acetylene flame. The instrumental parameters are shown in Table 1.

Reagents. The concentrated acids, HNO₃ (Carlo Erba, Milano, Italy) and HF (Panreac, Barcelona, Spain), used for digestion were of analytical reagent grade. Deionized distilled water was used throughout. All glassware and plasticware were washed with 5% v/v nitric acid and rinsed with deionized distilled water.

Stock standard solutions of Cu, Zn, Mn, Fe, Mg, and Ca containing 1000 mg L⁻¹ of each element were obtained by dissolving the pure metals or their compounds (Merck, Darmstadt, Germany) in HNO₃, HCl (Merck), or their mixtures. Calibration standards of each element were obtained by appropriate dilution of the stock solutions.

Samples. In total, 22 different medicinal, aromatic, and seasoning plants were subjected to microwave digestion. The samples were obtained commercially as a powder or as leaves. The samples studied are shown in Table 2.

The samples were ground with a vibrational agate ball mill for a time between 2 and 5 min. The powdered samples were sieved to ensure a particle size <200 μm. Plant materials were dried for 24 h, at a temperature of 70 °C, prior to analysis. Once the plants were powdered and dried, they were stocked in capped polypropylene flasks inside a desiccator.

The CRM GBW07605 tea leaves (National Research Center for Certified Reference Materials, China) were used for validation purposes.

Digestion Procedures. The following general procedure was employed for wet ashing of samples: An amount of the powdered plant material was weighed into the PTFE vessel, the acid digestion agent was added, and then the reactor was closed and heated; the reactor was cooled in an ice bath before being opened; the contents of each vessel were heated to dryness and dissolved with 1 mL of HCl; the solution was quantitatively transferred into a 5 mL volumetric flask and made up to volume with deionized distilled water. A blank was treated in the same way.

Table 2. Plants Analyzed by FAAS Using Optimized Digestion Conditions

plant	scientific name	species	part of plant analyzed
birch	<i>Betula alba</i>	Betulaceae	leaf
garlic	<i>Allium sativum</i>	Liliaceae	bulb
basil	<i>Ocimum basilicum</i>	Lamiaceae	leaf
aniseed	<i>Pimpinella anisum</i>	Umbelliferae	seed
orange blossom	<i>Citrus sinensis</i>	Rutaceae	flower
cinnamon	<i>Cinnamomum zeylanicum</i>	Laureaceae	bark
clove	<i>Caryophyllus aromaticus</i>	Myrtaceae	flower
cumin	<i>Cominum odorum</i>	Umbelliferae	seed
dill	<i>Anethum graveolens</i>	Umbelliferae	seed
tarragon	<i>Artemisa absinthium</i>	Asteraceae	leaf
mint	<i>Mentha piperita</i>	Lamiaceae	leaf
nutmeg	<i>Myristica fragrans</i>	Myristiaceae	seed
oregano	<i>Origanum majorana</i>	Lamiaceae	leaf
parsley	<i>Petroselinum sativum</i>	Umbelliferae	leaf
sweet paprika	<i>Piper officinalis</i>	Myrtaceae	fruit
hot paprika	<i>Piper caspicum</i>	Myrtaceae	fruit
white pepper	<i>Piper nigrum</i>	Piperaceae	fruit
black pepper	<i>Piper nigrum</i>	Piperaceae	fruit
rosemary	<i>Rosmarinus officinalis</i>	Lamiaceae	leaf
sage	<i>Salvia officinalis</i>	Lamiaceae	leaf
black tea	<i>Thea sinensis</i>	Theaceae	leaf
thyme	<i>Thymus vulgaris</i>	Lamiaceae	leaf

Table 3. Assignment of Variables and Levels for the Digestion Methods

variable	level +	level –	optimum conditions
low-pressure digestion vessel			
A, nitric acid vol (mL)	5	2	5
B, hydrofluoric acid vol (mL)	0.5	0	0.5
C, microwave power (W)	540	300	300
D, digestion time (min)	15	5	5
E, predigestion	yes	no	yes
medium-pressure digestion vessel			
A, nitric acid vol (mL)	5	3	5
B, hydrofluoric acid vol (mL)	0.5	0	0.5
C, microwave power (W)	540	300	540
D, digestion time (min)	3	2	3
high-pressure digestion vessel			
A, nitric acid vol (mL)	10	5	5
B, hydrofluoric acid vol (mL)	1	0	1
C, digestion time (h)	2	1	1

Method I (Low-Pressure Reactor). About 0.5 g of sample was treated with 5 mL of 69.5% w/w HNO₃ and 0.5 mL of 48% w/w HF and digested in the CEM microwave oven under a preselected program [two stages of 1 min at 40 and 80 psi, respectively, and a final stage at 120 psi for 5 min (300 W power)].

Method II (Medium-Pressure Reactor). About 0.2 g of sample was treated with 5 mL of 69.5% w/w HNO₃ and 0.5 mL of 48% w/w HF and digested in the domestic microwave oven at 540 W for 3 min.

Method III (High-Pressure Reactor). A mass sample of 0.5 g was treated with 5 mL of 69.5% w/w HNO₃ and 1 mL of 48% w/w HF by heating in a hot plate at ~180 °C for 2 h.

Experimental Design. The Plackett–Burman fractional factorial design was used as a screening approach with the aim of establishing the significant factors that influence the digestion methods and selecting suitable digestion conditions (Araujo and Brereton, 1996). The application of this factorial design reduced the development time of the methods and provided less ambiguous digestion conditions, hence facilitating data interpretation.

The variables studied as well as the values for each (+ representing the maximum level and – the minimum level) are shown in Table 3. Maximum and minimum levels were chosen according to previous experiences.

The programmed experiments are summarized in the matrices shown in Table 4. The result of each experiment was the average value of three replicates.

Table 4. Plackett–Burman Experimental Matrices for Five, Four, and Three Variables

expt	variable					expt order	results
	A	B	C	D	E		
1	+	+	+	-	+	(8)	y_1
2	+	+	-	+	-	(5)	y_2
3	+	-	+	-	-	(4)	y_3
4	-	+	-	-	+	(7)	y_4
5	+	-	-	+	+	(3)	y_5
6	-	-	+	+	+	(2)	y_6
7	-	+	+	+	-	(6)	y_7
8	-	-	-	-	-	(1)	y_8
1	+	+	+	-		(8)	y_1
2	+	+	-	+		(5)	y_2
3	+	-	+	-		(4)	y_3
4	-	+	-	-		(7)	y_4
5	+	-	-	+		(3)	y_5
6	-	-	+	+		(2)	y_6
7	-	+	+	+		(6)	y_7
8	-	-	-	-		(1)	y_8
1	-	-	-			(3)	y_1
2	-	+	+			(4)	y_2
3	+	-	+			(2)	y_3
4	+	+	-			(1)	y_4

When the matrix for three variables was employed, the main effect of a variable (e.g., variable A) was calculated as follows:

$$\text{effect of A} = (y_3 + y_4)/2 - (y_1 + y_2)/2 \quad (1)$$

When the matrices for five and four variables were employed, the main effect of a variable was calculated as follows:

effect of A =

$$(y_1 + y_2 + y_3 + y_5)/4 - (y_4 + y_6 + y_7 + y_8)/4 \quad (2)$$

A main effect was statistically significant when it was greater than twice the average standard deviation (\bar{s}). The positive level of the variable was chosen when the result was positive and vice versa.

Analytical Determinations. Three subsamples of each material were digested using method I. With each series of digestions a blank was included. All measurements were run in triplicate for the sample and standard solutions. Determination of metal contents in samples was based on a calibration graph obtained from standard solutions.

RESULTS AND DISCUSSION

Optimization Study. Birch, cinnamon, and orange blossom were used for optimization purposes. The analytical results obtained for the three samples in each experiment are shown in Tables 5, 6, and 7 for cinnamon, birch, and orange blossom, respectively. The effects of all variables, calculated by applying eqs 1 and 2, are shown in Tables 8–10.

Effect of Nitric Acid. The HNO_3 volume caused a positive effect on the determination of Cu, Zn, Fe, and Mg when the low-pressure reactor was used and on Zn, Fe, and Mg when the medium-pressure reactor was used. In the case of the high-pressure digestion reactor, the HNO_3 volume did not show any effect on metal determination. For the low- and medium-pressure reactors the optimum HNO_3 volume was 5 mL (i.e., the maximum level of this variable). In the high-pressure reactor the HNO_3 volume did not cause any effect in the range studied (i.e., 5–10 mL), so we chose the minimum level (i.e., 5 mL), which implied a low consumption of acid.

Effect of Hydrofluoric Acid. The HF volume showed a positive effect in the three digestion methods. Thus,

Table 5. Analytical Results for Cinnamon Using the Plackett–Burman Factorial Design^a

expt	Cu	Zn	Mn	Fe	Mg	Ca
Low-Pressure Reactor						
1	9.2 ± 0.3	26.7 ± 0.6	487 ± 0	381 ± 3	1499 ± 10	7893 ± 92
2	10.2 ± 0.3	26.6 ± 0.6	488 ± 1	377 ± 3	1506 ± 1	7934 ± 84
3	8.8 ± 0.3	25.8 ± 0.8	487 ± 1	344 ± 4	1499 ± 13	7942 ± 75
4	8.4 ± 0.3	26.6 ± 0.6	488 ± 2	353 ± 3	1475 ± 21	7983 ± 101
5	8.0 ± 0.3	22.9 ± 0.7	487 ± 1	303 ± 0	1482 ± 11	8004 ± 102
6	8.4 ± 0.2	21.4 ± 0.9	487 ± 1	306 ± 4	1441 ± 14	7898 ± 96
7	8.8 ± 0.3	24.7 ± 0.7	487 ± 1	306 ± 3	1423 ± 11	7653 ± 85
8	8.0 ± 0.2	22.9 ± 0.7	487 ± 1	301 ± 3	1483 ± 10	7754 ± 99
\bar{s}	0.27	0.70	1.0	2.9	11.4	91.8
Medium-Pressure Reactor						
1	<i>b</i>	25.8 ± 0.2	500 ± 4	386 ± 7	1485 ± 14	7960 ± 81
2	<i>b</i>	25.8 ± 0.1	500 ± 4	386 ± 8	1466 ± 12	7905 ± 75
3	<i>b</i>	23.2 ± 0.2	494 ± 4	341 ± 8	1394 ± 13	7794 ± 118
4	<i>b</i>	25.1 ± 0.2	494 ± 4	386 ± 8	1468 ± 13	7954 ± 106
5	<i>b</i>	23.9 ± 0.2	493 ± 4	329 ± 8	1419 ± 26	7814 ± 85
6	<i>b</i>	23.1 ± 0.0	500 ± 4	330 ± 8	1431 ± 12	7954 ± 95
7	<i>b</i>	25.9 ± 0.0	494 ± 5	386 ± 8	1466 ± 11	8102 ± 94
8	<i>b</i>	19.8 ± 0.3	488 ± 4	239 ± 8	1265 ± 13	7630 ± 100
\bar{s}		0.15	4.1	6.9	14.2	94.3
High-Pressure Reactor						
1	8.3 ± 0.3	24.4 ± 0.6	483 ± 3	346 ± 1	1419 ± 8	7691 ± 92
2	8.8 ± 0.0	25.3 ± 0.7	481 ± 1	344 ± 4	1394 ± 3	7809 ± 86
3	8.2 ± 0.3	24.2 ± 0.7	480 ± 0	338 ± 3	1398 ± 10	7984 ± 105
4	8.8 ± 0.0	22.7 ± 0.1	480 ± 2	355 ± 1	1430 ± 16	7743 ± 95
\bar{s}	0.15	0.52	1.5	2.2	9.2	94.5

^a Mean value \pm standard deviation ($n = 3$) ($\mu\text{g g}^{-1}$). ^b Not detected.

Table 6. Analytical Results for Birch Using the Plackett–Burman Factorial Design^a

expt	Cu	Zn	Mn	Fe	Mg	Ca
Low-Pressure Reactor						
1	7.2 ± 0.2	96.5 ± 1.0	905 ± 2	502 ± 8	4356 ± 25	6382 ± 60
2	8.1 ± 0.3	94.1 ± 1.3	904 ± 3	495 ± 5	4354 ± 14	6491 ± 67
3	6.9 ± 0.3	92.3 ± 2.6	908 ± 5	470 ± 4	4327 ± 33	6452 ± 59
4	6.9 ± 0.2	92.5 ± 0.7	907 ± 5	486 ± 6	4295 ± 12	6442 ± 61
5	6.5 ± 0.3	86.6 ± 2.0	905 ± 4	425 ± 8	4293 ± 41	6397 ± 72
6	6.8 ± 0.2	85.8 ± 1.5	906 ± 2	416 ± 5	4185 ± 10	6265 ± 54
7	6.3 ± 0.3	90.7 ± 1.9	903 ± 2	417 ± 6	4166 ± 17	6256 ± 42
8	6.2 ± 0.2	87.6 ± 0.4	902 ± 3	414 ± 5	4295 ± 19	6424 ± 63
\bar{s}	0.25	1.4	3.2	5.9	21.4	59.8
Medium-Pressure Reactor						
1	<i>b</i>	92.6 ± 1.3	910 ± 3	496 ± 6	4366 ± 21	6505 ± 35
2	<i>b</i>	92.3 ± 0.6	909 ± 4	493 ± 7	4321 ± 30	6496 ± 69
3	<i>b</i>	88.9 ± 0.8	908 ± 4	479 ± 5	4253 ± 14	6399 ± 73
4	<i>b</i>	92.1 ± 1.1	905 ± 5	499 ± 6	4263 ± 15	6475 ± 67
5	<i>b</i>	90.0 ± 0.4	903 ± 4	456 ± 6	4177 ± 20	6423 ± 56
6	<i>b</i>	89.4 ± 0.5	905 ± 3	455 ± 6	4233 ± 17	6487 ± 42
7	<i>b</i>	91.3 ± 2.5	906 ± 3	495 ± 8	4301 ± 31	6502 ± 58
8	<i>b</i>	80.3 ± 1.3	901 ± 3	326 ± 5	4096 ± 14	6305 ± 44
\bar{s}		1.1	3.6	6.1	20.2	55.5
High-Pressure Reactor						
1	6.4 ± 0.2	88.3 ± 0.5	905 ± 3	485 ± 3	4325 ± 21	6304 ± 61
2	6.9 ± 0.2	90.6 ± 1.6	908 ± 2	483 ± 6	4223 ± 35	6383 ± 52
3	6.2 ± 0.1	86.5 ± 0.7	906 ± 4	471 ± 4	4237 ± 24	6459 ± 49
4	7.0 ± 0.3	93.4 ± 0.9	908 ± 3	495 ± 5	4296 ± 12	6426 ± 43
\bar{s}	0.20	0.9	3.0	4.5	26.8	51.2

^a Mean value \pm standard deviation ($n = 3$) ($\mu\text{g g}^{-1}$). ^b Not detected.

the effect of HF volume was positive on Cu, Zn, and Fe when the low- and high-pressure reactors were used. Likewise, a positive effect was observed for Zn, Fe, and Mg with the medium-pressure reactor. As consequence, the maximum level of this variable was chosen in the three digestion methods.

Effect of Microwave Power. This variable was studied for the low- and medium-pressure reactors. Whereas in the low-pressure reactor this variable had a negative effect on Mg, in the case of the medium-pressure reactor it had a positive effect on Zn, Fe, and

Table 7. Analytical Results for Orange Blossom Using the Plackett–Burman Factorial Design^a

expt	Cu	Zn	Mn	Fe	Mg	Ca
Low-Pressure Reactor						
1	5.0 ± 0.2	25.6 ± 0.5	19.5 ± 0.3	354 ± 9	2305 ± 10	5959 ± 65
2	5.1 ± 0.2	25.6 ± 0.5	19.6 ± 0.2	347 ± 10	2310 ± 11	6084 ± 39
3	4.8 ± 0.2	24.3 ± 0.5	18.9 ± 0.2	322 ± 9	2300 ± 9	6051 ± 56
4	4.4 ± 0.2	25.7 ± 0.6	19.1 ± 0.3	329 ± 8	2278 ± 13	6112 ± 71
5	4.1 ± 0.2	20.3 ± 0.5	18.5 ± 0.3	286 ± 9	2289 ± 10	6035 ± 72
6	4.6 ± 0.2	19.8 ± 0.5	19.0 ± 0.3	289 ± 8	2237 ± 12	5931 ± 66
7	4.8 ± 0.2	23.6 ± 0.5	19.7 ± 0.2	291 ± 9	2218 ± 12	5895 ± 49
8	4.2 ± 0.2	20.8 ± 0.4	19.4 ± 0.4	296 ± 9	2283 ± 11	5783 ± 66
s	0.20	0.50	0.28	8.9	11.2	60.5
Medium-Pressure Reactor						
1	<i>b</i>	24.5 ± 0.3	19.9 ± 0.2	359 ± 6	2292 ± 12	6043 ± 43
2	<i>b</i>	24.7 ± 0.3	20.0 ± 0.2	356 ± 5	2258 ± 13	5989 ± 51
3	<i>b</i>	20.9 ± 0.3	19.6 ± 0.2	323 ± 4	2121 ± 14	5896 ± 66
4	<i>b</i>	23.8 ± 0.3	19.5 ± 0.3	361 ± 3	2265 ± 14	6073 ± 59
5	<i>b</i>	21.2 ± 0.4	19.6 ± 0.2	308 ± 3	2196 ± 13	5925 ± 72
6	<i>b</i>	20.3 ± 0.3	19.7 ± 0.2	305 ± 5	2219 ± 11	6017 ± 46
7	<i>b</i>	24.6 ± 0.3	19.5 ± 0.3	355 ± 4	2266 ± 9	6103 ± 51
8	<i>b</i>	18.0 ± 0.3	19.3 ± 0.2	241 ± 2	1997 ± 15	5812 ± 59
s		0.31	0.22	4.1	12.6	55.9
High-Pressure Reactor						
1	4.7 ± 0.1	22.5 ± 0.5	20.0 ± 0.2	341 ± 6	2256 ± 15	5896 ± 49
2	4.9 ± 0.1	22.9 ± 0.5	20.1 ± 0.3	345 ± 7	2245 ± 14	5935 ± 38
3	4.4 ± 0.1	20.8 ± 0.4	19.8 ± 0.2	326 ± 5	2231 ± 15	6027 ± 53
4	5.1 ± 0.1	23.6 ± 0.5	19.7 ± 0.1	359 ± 5	2282 ± 14	5923 ± 56
s	0.1	0.47	0.20	5.7	14.5	49.0

^a Mean value ± standard deviation ($n = 3$) ($\mu\text{g g}^{-1}$). ^b Not detected.

Table 8. Effect of the Selected Variables for Digestion of Cinnamon

	Cu	Zn	Mn	Fe	Mg	Ca
Low-Pressure Vessel						
A	0.6 ^a	1.6 ^a	0.1	34.7 ^a	41.3 ^a	121.0
B	0.8 ^a	2.9 ^a	0.4	40.4 ^a	-0.3	-33.7
C	0.2	-0.2	-0.2	0.8	-20.8 ^a	-72.2
D	0.2	-1.6 ^a	0.2	-21.7 ^a	-26.1 ^a	-20.8
E	-0.4	0.6	-0.0	4.0	-3.4	123.8
Medium-Pressure Vessel ^b						
A		1.8 ^a	2.5	25.2 ^a	33.3 ^a	-41.8
B		2.5 ^a	2.8	76.7 ^a	94.2 ^a	182.1
C		0.8 ^a	2.9	25.5 ^a	39.3 ^a	126.5
D		1.8 ^a	2.6	19.6 ^a	42.6 ^a	109.7
High-Pressure Vessel						
A	-0.0	0.1	-2.1	1.5	7.5	113.5
B	0.6 ^a	1.2 ^a	-0.9	7.1 ^a	3.2	-61.5
C	-0.4 ^a	-1.1 ^a	1.2	-12.0 ^a	-26.4 ^a	179.5

^a Values higher than the experimental error (2s). ^b Cu could not be detected by FAAS under conditions employed in method II.

Mg. Therefore, in the first case we chose the minimum level of microwave power (i.e., 300 W) and, in the second case, the maximum level (i.e., 540 W).

Effect of Digestion Time. This variable had a negative effect on Zn, Fe, and Mg, with the low-pressure reactor, so the minimum level was selected. In the medium-pressure reactor this variable had a positive effect on Zn, Fe, and Mg, so the maximum level was selected. Finally, with the high-pressure reactor, the digestion time had a negative effect on Zn, Fe, and Mg and also for Cu in the cinnamon and orange blossom samples, so the minimum level was selected.

Effect of Predigestion. This variable was investigated with only the low-pressure reactor. Predigestion had no effect on metal content found, but it was used so that acid attack over vessels was minimized.

The optimum conditions selected for each studied digestion method are also included in Table 3 (third column).

Table 9. Effect of the Selected Variables for Digestion of Birch

	Cu	Zn	Mn	Fe	Mg	Ca
Low-Pressure Vessel						
A	0.6 ^a	3.2 ^a	1.3	39.8 ^a	97.3 ^a	83.7
B	0.5 ^a	5.4 ^a	-0.5	43.9 ^a	17.8	8.2
C	-0.1	1.1	1.1	-3.4	-50.5 ^a	-99.6
D	0.1	-2.9 ^a	-1.2	-29.7 ^a	-68.6 ^a	-72.8
E	0.1	-0.8	1.5	8.3	-3.2	-36.5
Medium-Pressure Vessel ^b						
A		2.7 ^a	3.2	37.4 ^a	56.1 ^a	13.7
B		4.9 ^a	3.2	66.5 ^a	123.0 ^a	91.2
C		1.8	2.7	37.8 ^a	74.6 ^a	48.5
D		2.3 ^a	-0.3	24.7 ^a	13.8	56.3
High-Pressure Vessel						
A	-0.1	0.5	0.5	-1.1	-8.0	99.1
B	0.7 ^a	4.6 ^a	2.5	11.3 ^a	-21.5	23.4
C	-0.2	-2.3 ^a	0.6	-13.0 ^a	-80.6 ^a	56.3

^a Values higher than the experimental error (2s). ^b Cu could not be detected by FAAS under conditions employed in method II.

Table 10. Effect of the Selected Variables for Digestion of Orange Blossom

	Cu	Zn	Mn	Fe	Mg	Ca
Low-Pressure Vessel						
A	0.5 ^a	1.5 ^a	-0.2	26 ^a	47.3 ^a	102.1
B	0.4 ^a	3.8 ^a	0.5	32 ^a	0.5	62.5
C	0.3	0.2	0.1	-0.5	-25.1 ^a	-44.5
D	0.1	-1.7 ^a	0.0	-22 ^a	-28.5 ^a	10.2
E	-0.2	-0.7	-0.4	0.5	-0.6	56.0
Medium-Pressure Vessel ^b						
A		1.1 ^a	0.3	21.0 ^a	30.7 ^a	-38.3
B		4.3 ^a	0.2	63.4 ^a	137.0 ^a	95.0
C		0.7 ^a	0.1	19.1 ^a	45.5 ^a	65.4
D		0.9 ^a	0.1	10.4 ^a	66.1 ^a	52.5
High-Pressure Vessel						
A	0.0	-0.5	-0.3	-0.5	5.9	59.2
B	0.5 ^a	1.6 ^a	0.0	18.5 ^a	20.0	-32.6
C	-0.3 ^a	-1.2 ^a	0.1	-14.5 ^a	-31.4 ^a	71.2

^a Values higher than the experimental error (2s). ^b Cu could not be detected by FAAS under conditions employed in method II.

Table 11. Results for the Cinnamon Sample Obtained with the Three Digestion Methods^a

metal	pressure vessel		
	low (method I)	medium ^b (method II)	high (method III)
Cu	8.4 ± 0.3		8.7 ± 0.2
Zn	23.4 ± 0.0	24.0 ± 0.2	24.3 ± 0.6
Mn	495 ± 10	495 ± 6	488 ± 4
Fe	359 ± 2	368 ± 4	367 ± 6
Mg	1435 ± 19	1468 ± 21	1459 ± 17
Ca	8082 ± 132	7988 ± 58	7993 ± 85

^a Average concentration ± standard deviation ($p = 0.05$ and $n = 3$) ($\mu\text{g g}^{-1}$). ^b Cu could not be detected under the conditions employed in method II.

Comparison of Digestion Methods. The three optimized methods were applied to the digestion of the cinnamon sample. The results obtained for three replicate determinations are shown in Table 11.

One-way ANOVA for each determined element was performed with the Statview TM SE + Graphics computer program. ANOVA analysis showed that no significant differences occurred at the 95% confidence level.

The main differences among the three digestion methods lie in the time required to complete the digestion and in safety. Microwave digestions performed with method I (i.e., controlled-pressure reactor) had

Table 12. Validation of the Proposed Digestion Method with Low-Pressure Vessel against CRM GBW 07605 Tea Leaves^a

element	certified value	found value	relative error (%)
Cu	17.3 ± 1.0	17.0 ± 0.4	-1.7
Zn	26.3 ± 0.9	26.8 ± 0.6	+1.9
Mn	1240 ± 40	1256 ± 17	+1.3
Fe	264 ± 10	260 ± 5	-1.5
Mg ^b	0.17 ± 0.01	0.16 ± 0.01	-5.9
Ca ^b	0.43 ± 0.02	0.40 ± 0.04	-7.0

^a Average concentration ± confidence interval ($p = 0.05$ and $n = 5$) ($\mu\text{g g}^{-1}$). ^b Metal concentration expressed as mg g^{-1} .

some advantages: it was the safest digestion procedure; it allowed the simultaneous dissolution of up to 12 samples; and the digestion processes were developed in successive steps until completion, which extended the digestion vessel lifetime. Therefore, this method was employed for subsequent analysis of plant samples.

Validation of Method I. The proposed digestion method was validated against the CRM GBW07605 tea plant. The experimental results obtained for Cu, Zn, Mn, Fe, Mg, and Ca are shown in Table 12. In all cases, a good agreement between the certified and found concentration values was found. When the t test for comparison of an experimental value with the certified value was applied, t_{exp} was less than t_{crit} ($p = 0.05$, $n = 5$) in all cases, hence meaning that the null hypothesis could be accepted. The relative error ranged from -7% for Cd to +1.9 for Zn. The proposed digestion method led to sufficiently accurate and reproducible results.

Analysis of Samples. A total of six elements (i.e., Cu, Zn, Mn, Fe, Mg, and Ca) were determined in the powdered plant samples by flame-atomic absorption spectrometry (FAAS) with previous acid digestion with method I. Metal contents for each sample are shown in Table 13. The Cu concentration levels ranged from 4 to 35 $\mu\text{g g}^{-1}$, most samples having contents between 5 and 15 $\mu\text{g g}^{-1}$. Sage had the lowest Cu concentration and black tea the highest. Zn concentration values varied from 7 to 90 $\mu\text{g g}^{-1}$, with values frequently in the range 20–40 $\mu\text{g g}^{-1}$. Black pepper had the lowest Zn concentration, whereas birch had the highest. The Mn concentration levels varied from 9 to 939 $\mu\text{g g}^{-1}$, with values

frequently in the 25–325 $\mu\text{g g}^{-1}$ interval. The lowest Mn concentration value was obtained for garlic, whereas the highest one was obtained for clove. Most of the samples analyzed had Fe concentration values ranging from 33 to 2486 $\mu\text{g g}^{-1}$ with the exception of mint, which had an Fe concentration of 6588 $\mu\text{g g}^{-1}$. The high concentration value found in this sample may be due to contamination processes occurring during collection, drying, and packing operations, because this metal is included in the composition of most machines and tools. The Mg concentration levels ranged from 495 to 7458 $\mu\text{g g}^{-1}$, with values frequently between 2000 and 4500 $\mu\text{g g}^{-1}$. Garlic had the lowest Mg concentration and basil the highest. Finally, the Ca concentration levels were in the range 386–21500 $\mu\text{g g}^{-1}$. As in most cases, garlic had the lowest Ca concentration and, again, basil the highest.

As was expected, Ca and Mg showed the highest concentration levels in the plant samples, whereas the lowest concentration values were observed for Cu. Likewise, the highest metal contents were mainly found in leaf, flower, and seed, in which most nutrients were located (Chizzola and Franz, 1996; Markert, 1993). On the contrary, garlic, the only sample analyzed corresponding to a bulb, usually contained the lowest metal concentration.

Conclusions. The three digestion methods described offered an efficient sample preparation for direct metal detection by FAAS: (i) their application can be extended to a great variety of plant samples; (ii) different metals can be determined under the same digestion conditions; (iii) the time required for sample digestion is shortened when using microwave energy; and (iv) screening factorial design offers an efficient and convenient strategy for digestion optimization. Acid digestion performed with a controlled-pressure reactor (low pressure) provides increased safety in comparison with medium-pressure reactors heated in a domestic microwave oven.

Together with the already published data (Majid et al., 1995; Chizzola and Franz, 1996; González Soto et al., 1996; Berthelsen et al., 1995) the reported results should contribute to the establishment of regular levels of minor and trace elements in plants. Metal contents

Table 13. Metal Content of the Analyzed Samples^a

plant	Cu	Zn	Mn	Fe	Mg	Ca
birch	6.7 ± 0.2	89.3 ± 1.5	903 ± 1	483 ± 10	4251 ± 58	6438 ± 55
garlic	4.2 ± 0.2	21.8 ± 0.6	8.6 ± 0.3	33.5 ± 1.3	495 ± 10	386 ± 5
basil	18.5 ± 0.8	39.5 ± 1.0	114 ± 3	667 ± 7	7458 ± 153	21.4 ± 0.4 ^b
aniseed	10.1 ± 0.4	53.6 ± 0.5	41.0 ± 1.2	450 ± 4	2531 ± 50	5725 ± 85
orange blossom	4.5 ± 0.1	21.4 ± 0.4	19.9 ± 0.2	330 ± 7	2244 ± 16	5925 ± 58
cinnamon	8.4 ± 0.2	23.4 ± 0.0	495 ± 10	359 ± 2	1435 ± 19	8082 ± 132
clove	5.6 ± 0.4	14.5 ± 0.6	939 ± 15	219 ± 7	3309 ± 65	10.0 ± 0.1 ^b
cumin	8.6 ± 0.4	33.4 ± 0.4	60.2 ± 0.7	1369 ± 24	3874 ± 73	5442 ± 18
dill	14.4 ± 0.4	46.9 ± 1.1	123 ± 1	2486 ± 28	3007 ± 9	8256 ± 101
tarragon	6.4 ± 0.2	35.8 ± 1.1	156 ± 4	229 ± 4	1333 ± 36	8768 ± 181
mint	18.0 ± 0.3	33.2 ± 0.9	313 ± 15	6588 ± 138	6169 ± 46	8226 ± 83
nutmeg	11.7 ± 0.7	19.6 ± 0.6	42.7 ± 0.1	109 ± 3	2218 ± 42	1488 ± 53
oregano	7.5 ± 0.4	37.5 ± 1.2	60.5 ± 0.6	742 ± 27	3793 ± 70	18.5 ± 0.3 ^b
parsley	6.5 ± 0.3	31.4 ± 0.8	108 ± 1	159 ± 2	2799 ± 56	11.9 ± 0.2 ^b
sweet paprika	12.7 ± 0.3	29.2 ± 0.8	26.0 ± 0.8	440 ± 5	2444 ± 32	1551 ± 55
hot paprika	10.6 ± 0.4	33.9 ± 0.4	29.8 ± 1.1	703 ± 24	2927 ± 73	2217 ± 15
white pepper	6.8 ± 0.3	11.1 ± 0.4	55.6 ± 1.6	103 ± 3	808 ± 28	1887 ± 40
black pepper	9.1 ± 0.1	6.9 ± 0.1	244 ± 7	640 ± 7	1971 ± 19	2383 ± 46
rosemary	7.7 ± 0.4	27.6 ± 1.4	41.6 ± 0.6	999 ± 28	2988 ± 56	15.8 ± 0.2 ^b
sage	4.2 ± 0.2	18.6 ± 0.7	41.4 ± 1.2	773 ± 13	4531 ± 84	21.3 ± 0.4 ^b
black tea	34.6 ± 1.2	40.1 ± 0.8	735 ± 13	304 ± 10	2458 ± 44	1784 ± 32
thyme	7.9 ± 0.6	43.6 ± 1.1	128 ± 5	815 ± 20	3476 ± 34	16.1 ± 0.3 ^b

^a Average concentration ± standard deviation ($n = 3$) ($\mu\text{g g}^{-1}$). ^b Average concentration expressed as mg g^{-1} .

of Cu, Zn, Mn, Fe, Mg, and Ca in 22 plants found in the market for human consumption were established.

LITERATURE CITED

- Abu-Samra, A.; Steven, J.; Koirtyohann, S. R. Wet ashing of some biological samples in microwave oven. *Anal. Chem.* **1975**, *47*, 1475–1477.
- Araujo, P. W.; Brereton, R. G. Experimental design I. Screening. *Trends Anal. Chem.* **1996**, *15*, 26–31.
- Berthelsen, B. O.; Steinnes, E.; Solberg, W.; Jingsen, L. Heavy metal concentrations in plants in relation with atmospheric heavy metal deposition. *J. Environ. Qual.* **1995**, *24*, 1018–1026.
- Blanco, M. J.; Ribó, R.; Tomás, X.; Obiols, J. Validation of microwave dissolution of lyophilized vegetal tissue for the subsequent determination of copper, lead, chromium and cadmium by using atomic absorption spectrometry. *Anal. Proc. Incl. Anal. Commun.* **1994**, *31*, 353–356.
- Carlosena, A.; Prada, D.; Fernández, E. Extracción de elementos metálicos de material de referencia vegetal (hojas de olivo) con energía de microondas y ácido nítrico-peróxido de hidrógeno. *Quim. Anal.* **1994**, *13*, 214–217.
- Chizzola, R.; Franz, Ch. Metallic trace elements in medicinal and aromatic plants from Austria. *Angew. Bot.* **1996**, *70*, 52–56.
- González Soto, E.; Alonso Rodríguez, E.; López Mahía, P.; Muniategui Lorenzo, S.; Prada Rodríguez, D. Determination of trace elements in tree leaves. *Ann. Chim.* **1996**, *86*, 181–191.
- Kojima, I.; Uchida, T.; Iida, C. Pressurized microwave digestion of biological samples for metal determination. *Anal. Sci.* **1988**, *4*, 211–214.
- Lavilla, I.; Pérez Cid, B.; Bendicho, C. Optimization of digestion methods for sewage sludge using the Plackett-Burman saturated design. *Fresenius' J. Anal. Chem.* **1998**, *361*, 164–167.
- Majid, A. Ab.; Sarmani, S.; Yusoff, N. I.; Wei, Y. K.; Hamzah, F. Trace elements in Malaysian medicinal plants. *J. Radioanal. Nucl. Chem., Articles* **1995**, *195*, 173–183.
- Markert, B. *Plants as Biomonitors. Indicators for Heavy Metals in the Terrestrial Environment*; Markert, B., Ed.; VCH: Berlin, Germany, 1993.
- Matejovic, I.; Durackova, A. Comparison of microwave digestion, wet and dry mineralization and solubilization of plant sample for determination of calcium, magnesium, potassium, phosphorus, sodium, iron, zinc, copper, and manganese. *Commun. Soil Sci. Plant Anal.* **1994**, *25*, 1277–1288.
- Mingorance, M. D.; Pérez-Vázquez, M. L.; Lachica, M. Microwave digestion methods for the atomic spectrometric determination of some elements in biological samples. *J. Anal. At. Spectrom.* **1993**, *8*, 853–858.
- Mohd, A. A.; Dean, J. R.; Tomlinson, W. R. Factorial design approach to microwave dissolution. *Analyst* **1992**, *117*, 1743–1748.
- Smith, F. E.; Arsenault, E. A. Microwave-assisted sample preparation in analytical chemistry. *Talanta* **1996**, *43*, 1207–1268.

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