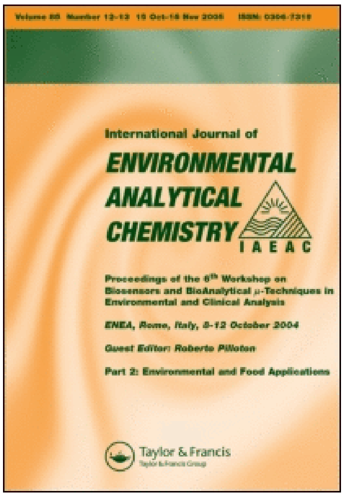

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Comparison of wet microwave digestion methods of plant materials for the determination of metals by flame atomic absorption spectrometry

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COMPARISON OF WET MICROWAVE DIGESTION METHODS OF PLANT MATERIALS FOR THE DETERMINATION OF METALS BY FLAME ATOMIC ABSORPTION SPECTROMETRY

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Microwave closed-system wet digestion procedures for plant samples were examined. Each procedure was tested with samples of tobacco and cabbage, and included digestion by the use of different acids composition, almost complete evaporation of the digest, and then dissolution of the residue in 1% nitric acid. Three microwave digestion programs that varied power, duration, and temperature were used. Closed-vessel reactions followed open-vessel reaction-delay time. Using flame atomic absorption spectrometry on the digests, four or five elements were determined to evaluate effectiveness, precision and accuracy of analytes extraction into solution. After a preliminary study of tobacco digests, the four most effective procedures were chosen, and detailed investigations were carried out on both tobacco and cabbage reference materials. Although all four of the final procedures were accurate, the most precise procedure, with the lowest errors of determination, was using reverse 'aqua regia' for tobacco and 'aqua regia' for cabbage.

Keywords: Digestion; Wet microwave procedures; Plant materials; Flame atomic absorption spectrometry

INTRODUCTION

Decomposition procedures have been developed [1–2] and optimized for many kinds of samples including plant materials, in order to transfer the elements into solution, and to determine them using atomic spectrometry [3–5]. When using wet methods, in some analyses interference effects related to the type of acid can appear [6,7]. Over the last three decades, many of the wet methods have been adapted to microwave digestion techniques and are considered to be very effective [8–13]. Several others are still being examined [14–16] and validated [8,17]. General problems with environmental

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sample analysis have been recently summarized [18]. Hoenig *et al.* [19] also discuss in detail the fundamentals of the plant sample digestion. The authors emphasized problems of silica influencing the determination and compared three procedures that ensured the removal of Si. The wet procedures described in the literature differ slightly, and it is difficult to choose the best procedure for a multi-element analysis from a single digest [19,20].

Some of Hoenig's critical remarks could not be confirmed by the collective long-term experience of the authors of this paper. In many cases, undissolved sample residues do not affect the accuracy of determinations, although sometimes they can potentially contain analyte traces. Thus, the aim of this work was to provide additional information on plant-digestion procedures, based on the comparison and evaluation of a few commonly used wet microwave digestion methods, as applied to tobacco and cabbage. The effectiveness and precision of analytes transferring into solution were evaluated. Five elements, calcium (main component), magnesium and iron (minor constituents), and copper and zinc (trace elements), were determined at different concentrations in the plant materials.

EXPERIMENTAL

Plant Materials

Investigations were carried out for two plant materials: tobacco leaves and cabbage. Laboratory samples were prepared for both plants according to the preparation procedure provided with reference material for tobacco and cabbage, in the following way:

Approximately 2.5 kg of tobacco leaves, the variety called 'Milenium' (in Polish) grown in Poland near Krakow, were collected by hand and dried at ambient temperature. The leaves were crushed by hand, veins were removed, and the material was dried for 3 days at 70°C. The dried material was ground using an agate mortar and then additionally dried at 110°C and ground using a coffee mill.

The white cabbage (*Brasica capitata*) was collected near Krakow, and external leaves and stems were removed. Remaining leaves were spread on filter paper and dried for 5 days at 85°C. Gradually, leaves were crushed and veins removed by hand. Finally, the material was ground in an agate mortar, dried at 110°C and ground using a coffee-mill.

Both samples (230–300 g) were divided and stored in polyethylene bottles for use as the 'internal quality control samples'. The final precision and accuracy were tested using adequate plant reference materials: Oriental Tobacco Leaves (SRM CTA-OTL-1), Institute of Nuclear Chemistry Technology, Warsaw, Poland, and Cabbage Leaves (RM CL-1) Faculty of Physics and Nuclear Techniques, University of Mining and Metallurgy, Krakow, Poland.

Apparatus

A microwave closed-system Uni Clever–BM1z (Plazmatronika, Poland) with a PTFE vessel was used for sample digestion. A three-stage decomposition program was used for all procedures listed in Table I. Each decomposition stage applied a different combination of microwave power, duration and pressure range: I – 60% microwave power, 5 min, pressure range 17–20 atm; II – 80% microwave power, 5 min, pressure range 27–30 atm; III – 100% microwave power, 10 min, pressure range 42–45 atm. The temperature reached 200°C during the third stage.

TABLE I Description of the tested procedures

Procedure	*Mixture of reagents* (mL)	*Delay time* (h)	*Digestion time* (min)[a]	*Max. pressure* (atm)
I	$6HNO_3$	0	30	45
II	$6HNO_3 + 1HF$	0	30	45
III	$6HNO_3 + 2H_2O_2$	0.5	30	45
IV	$6HNO_3 + 1HClO_4$	0.5	30	45
V	$1.5HNO_3 + 4.5HCl$ aqua regia	1	30	45
VI	$6HNO_3 + 1H_2O_2$	1	30	45
VII	$1.5HNO_3 + 4.5HCl$	24	30	45
VIII	$6HNO_3 + 1.5HClO_4 +$ 1.5HCl after mineralization	1.5	30	45
IX	$4.5HNO_3 + 1.5HCl$ reversed aqua regia	1.5	30	45
X	$6HNO_3 + 1HClO_4$	1.5	30	45

[a] After 20 min of active digestion, the system was left for 10 min to cool down and to allow the pressure decrease.

All elements considered were determined by the use of a 3100 Perkin Elmer flame atomic absorption spectrometer. Standard conditions of the determination were applied at wavelength of 422.7 nm for Ca, 285.2 nm for Mg, 248.3 nm for Fe, 324.8 for Cu and 213.9 nm for Zn. An ionization buffer was added for calcium. All elements were analysed in the linear range of adequate calibrations graphs: calcium after 500-fold dilution; magnesium after 250-fold dilution; iron after fivefold dilution; zinc and copper from bulk solution of the tobacco digests or calcium after 50-fold dilution; magnesium after 100-fold dilution; iron and zinc from bulk solution of the cabbage digests.

Reagents and Solutions

The following reagents were used: HNO_3 65%, HF 50%, HCl 36%, NaCl 'POCh' Poland, $HClO_4$ 70% 'LOBA Feinchemie AG' Fischamend, H_2O_2 30% 'Chempur' Poland. All reagents used were of analytical grade. Stock standard solutions of calcium, magnesium, iron, copper and zinc were prepared from 'Titrisol' Merck. For dilution, 1% HNO_3 (by volume) was used. The solution was prepared by dilution of the concentrated acid with redistilled water.

PROCEDURE

Digestion procedures – all using a closed microwave system and program described earlier plus reagent compositions listed in Table I – were evaluated using the results of digest analysis by flame atomic absorption spectrometry.

In each test procedure, three or six portions of samples were mineralized. Each test sample weighed approximately 0.5 g. For procedures III–X, after treating each sample with a mixture of reagents, the reaction vessel was left open for the mentioned period of time to allow them to react vigorously. The vessel was then closed and placed in the microwave oven system, thus commencing the main part of the digestion process. Parallel blank tests were prepared using the same reagents in the same quantities and following the same procedure.

After decomposition of the test portions, the digests were transferred into PTFE dishes, and they evaporated almost completely. The residues were dissolved in 1% HNO_3, transferred into volumetric flasks and analysed. For each element the mean, standard deviation, and confidence interval (at a confidence level 0.95) were calculated for the concentrations. The mean effectiveness of the digestion, taking into account results of the elements determinations, was tested using two-way analysis of variance, with repetitions. The significance of the differences between all the procedures was also tested individually for particular elements, using the method of one-way analysis of variance (the F-Snedecor test at significance level $\alpha = 0.05$) [21].

To evaluate the reference material analyses, the trueness of particular element determinations was compared determining the relative errors calculated using the following equation:

$$\text{error}_{\text{rel}} = \frac{|\bar{x} - x_{\text{w.cert.}}|}{x_{\text{w.cert.}}} \times 100\%,$$

where: $\bar{x}$ is the mean determined value of adequate element concentration for a given procedure, and $x_{\text{w.cert.}}$ is the corresponding certificated value.

The total errors of determinations using a specified digestion procedure were calculated as the sum of the particular relative errors. Digestions were observed very carefully, and remarks on digestion completeness, vigour of reaction and solubility of products were noted and used as additional criteria for the procedure evaluation.

RESULTS AND DISCUSSION

In the preliminary study, the 10 procedures specified in Table I were tested using the ‘internal quality control sample’ of tobacco. To compare the effectiveness of digestion of a particular procedure for all considered elements on their different concentration levels, their signals were normalized in the following way: for every element in a series of procedures, the highest signal was chosen and assumed as 100% transfer. Remaining values were recalculated relative to this value. Thus, concentrations of different orders can be compared, and then the general effectiveness of all analyte transfers into solution and factors influencing it can be evaluated. These normalizations were done in a preliminary study, analysing the ‘internal quality control sample’ with unknown but absolute values of concentration of the elements of interest.

The test results for precision and of analytes transfer into solution are illustrated in Figs 1 and 2, respectively. Based on these experimental results, the following general remarks can be made:

- the effectiveness of analytes extraction into solution significantly depends on the procedure tested, the elements considered and their interaction;
- the results of the calcium and magnesium determinations are significantly lower for procedure II than for the others;
- extension of the reaction time in an opened vessel for procedures VII and X as compared with procedures V and IV, respectively, improved the precision of digestion by reducing the vigour of the reaction after closing the vessel;
- the highest average concentrations were achieved when procedures VII–X were applied;
- for every procedure, sample residues were observed, the lowest for procedure IX.

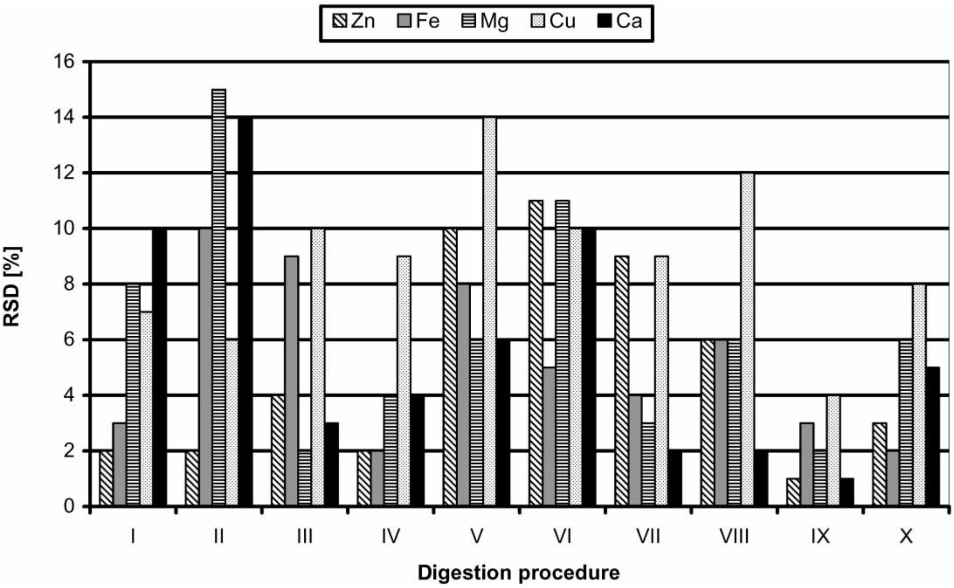

FIGURE 1 Relative standard deviation of the determination of Ca, Mg, Fe, Cu and Zn in the digest solutions from the tobacco.

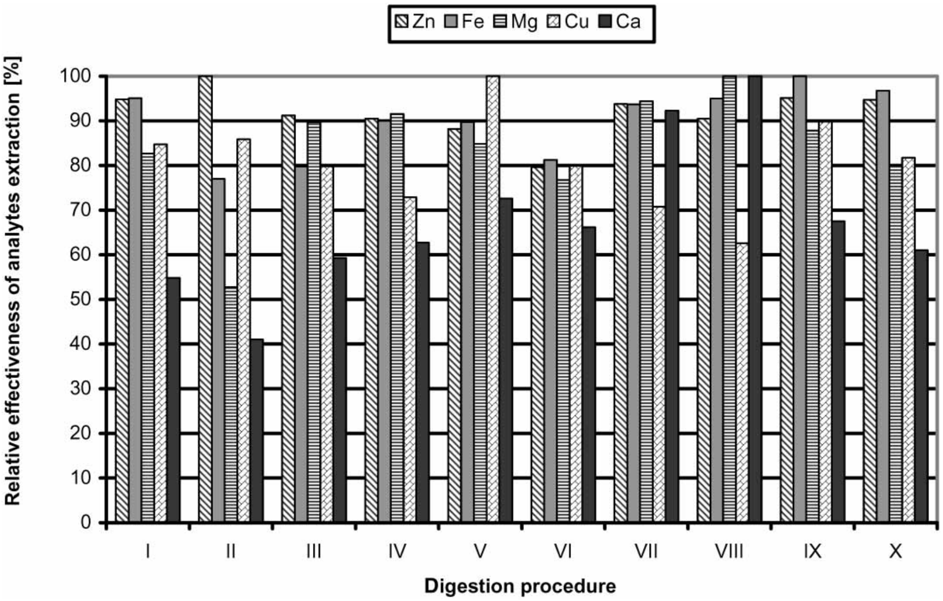

FIGURE 2 Relative effectiveness of Ca, Mg, Fe, Cu and Zn extraction from the internal laboratory standard of tobacco into solution.

On the basis of the preliminary study of the 10 procedures using tobacco digests, four procedures (VII to X) were selected and compared with the cabbage ‘internal quality control sample’. The results of the Ca, Mg, Fe and Zn determinations for the cabbage digests are presented in Figs 3 and 4. Concentrations of copper were below the

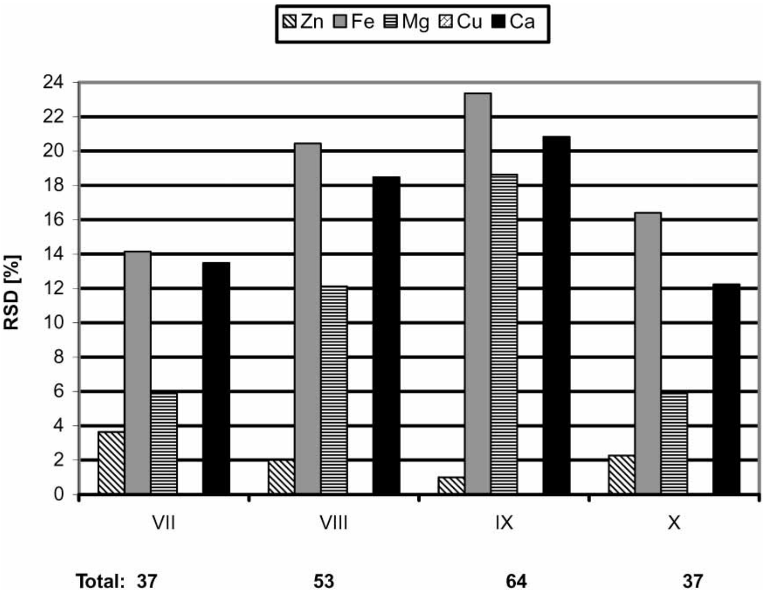

FIGURE 3 Relative standard deviation of the determination of Ca, Mg, Fe, Cu and Zn in the digest solutions from the cabbage 'internal quality control sample'.

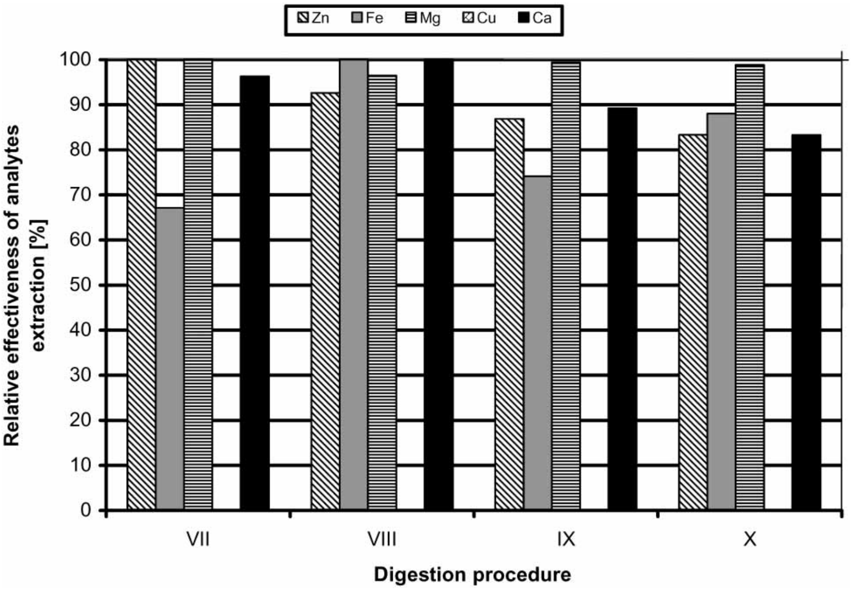

FIGURE 4 Relative effectiveness of Ca, Mg, Fe, Cu and Zn extraction from the 'internal quality control sample' of cabbage.

detection limits of the FAAS. The digestion of cabbage was complete (no residue was observed).

Procedures VII to X were also examined further against the tobacco and cabbage leaf reference materials. Two reference materials – SRM CTA-OTL-1 of tobacco and

RM CL-1 of cabbage – were analysed to evaluate the trueness of the determinations with the chosen digestion procedures. The results of these determinations are presented in Table II.

The evaluation of the trueness, expressed as a relative error of a particular element's determination and the total relative error, is presented in Fig. 5. The data presented in Table II and in Fig. 5 were calculated, based on the results of six replicates. These data show that the reference, determined mean values and confidence intervals coincide in most cases. The lowest total error of the determinations of Ca, Mg, Fe, Zn and Cu for tobacco was found when procedure IX was applied, whereas procedures VII and X were the most accurate for cabbage.

Although a very long (24 h) delay time was applied to the digestion of tobacco sample by the use of procedure VII, the reactions in the closed system were very vigorous,

TABLE II Results of the analysis of the reference materials

Element	*Ca* (mg/g)	*Mg* (mg/g)	*Fe* (μg/g)	*Zn* (μg/g)	*Cu* (μg/g)
Procedure: Tobacco SRM CTA-OTL-1					
VII	33.0 ± 2.4	4.66 ± 0.14	1089 ± 67	50.5 ± 1.7	15.4 ± 1.1
VIII	32.8 ± 2.1	4.54 ± 0.22	931 ± 99	44.9 ± 2.9	14.9 ± 0.4
IX	31.7 ± 3.3	4.74 ± 0.19	986 ± 48	45.7 ± 0.8	14.7 ± 0.4
X	29.8 ± 1.1	4.55 ± 0.35	850 ± 17	56.5 ± 3.4	14.2 ± 1.0
Ref. conc.	31.7 ± 1.2	4.47 ± 0.21	989[a]	49.9 ± 2.4	14.1 ± 0.5
Procedure: Cabbage RM CL-1					
VII	5.93 ± 0.9	1.69 ± 0.1	59.5 ± 9.5	41.1 ± 1.7	< D.L.[b]
VIII	5.41 ± 1.0	1.65 ± 0.2	45.5 ± 10.5	38.9 ± 1.8	< D.L.[b]
IX	5.76 ± 1.4	1.61 ± 0.3	38.1 ± 10.1	39.4 ± 2.4	< D.L.[b]
X	5.72 ± 0.8	1.68 ± 0.1	56.7 ± 10.5	39.6 ± 1.0	< D.L.[b]
Ref. conc.	6.25 ± 0.93	1.85 ± 0.13	58.4 ± 3.9	38.5 ± 1.9	3.3 ± 0.23

[a] Informative value. [b] The concentration of the digest was below the detection limit of determination by FAAS.

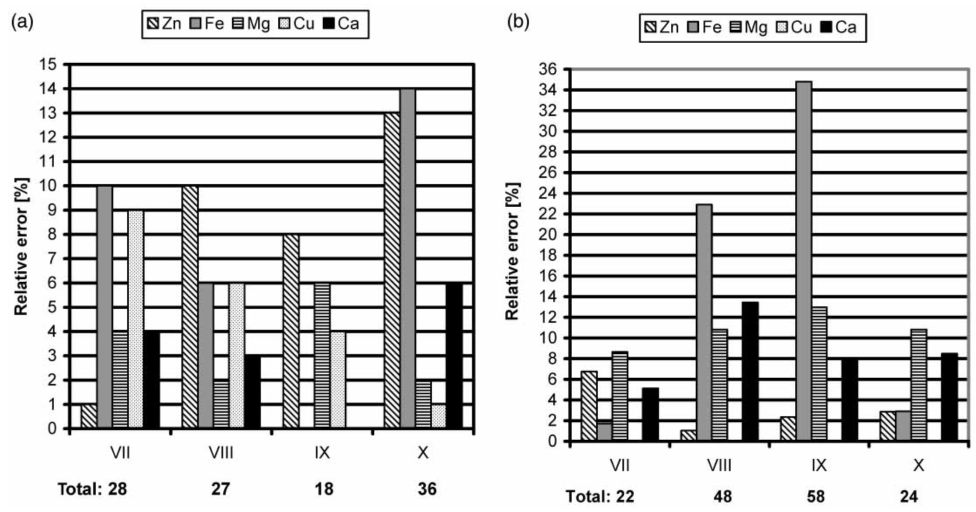

FIGURE 5 Relative errors of the Ca, Mg, Fe, Cu and Zn determination after digestion with the use of procedures VII–X: (a) SRM CTA-OTL-1 reference material of tobacco; (b) RM CL-1 reference material of cabbage.

and foaming was observed. In some cases, the foaming might lead to analyte losses or digest contamination from the stainless steel reactor lid; but for these procedures, the results of the determinations were accurate and precise. In all cases, the tobacco 'internal quality control sample' and the RM digestions, undigested residuals were noticed.

FIGURE OF MERIT

Accuracy

Analysis accuracy was determined in the second step only for procedures VII, VIII, IX and X, and was expressed in terms of trueness – relative to reference values. The confidence intervals of the determined concentrations of all elements and procedures considered in this step, except iron, coincide with the confidence intervals of the reference values. For iron in the tobacco SRM, no data about confidence interval are available (the concentration of iron is only informative). The confidence interval of the iron determination of the cabbage RM matched the reference values apart from procedure IX. The determination of iron exhibited a poor precision, thus increasing confidence intervals, which were probably significantly different from that of the reference value. Unfortunately, exact statistical comparisons of mean values and precisions were impossible because of a lack of a number of replicates for the respective reference data.

Precision

Precision was evaluated primarily for the 'internal quality control sample' and then for the tobacco and cabbage RMs. Data are presented in Figs 1 and 3, and Table II. A cross-comparison of SDs and RSDs can be made for procedures VII–X only, for which the 'internal quality control sample' and reference materials of both tobacco and cabbage were analysed. Generally, the standard deviations depended on the determined elements. The highest precision was observed for the magnesium determinations, especially in the cabbage 'internal quality control sample'. Because of the concentration differences of particular elements between the plants, the reference material and the 'internal quality control sample' of the same class of plant, RSDs differ significantly for both plant and element. These were also partially dependent on the degree of digests dissolution before determinations. Nevertheless, the confidence intervals of the determination of elements of interest were generally of the same order of magnitude as those of reference data, except for calcium in the tobacco RM (about twice as high) and iron in cabbage RM (about three times as high).

Detection Limits

For the determinations of calcium, magnesium and iron, the digests were adequately diluted to meet the middle linear range of calibration plots for these elements. Zinc and copper for tobacco, or iron and zinc for cabbage were determined from bulk digest solutions. Only the concentrations of copper in the cabbage were below the determination limit (D.L.) when the digests were transferred into 25-mL volumetric flasks, and they bordered this value when the digests were transferred into 10-mL volumetric flasks. The experimentally determined D.L. for copper, defined as 10 times the standard deviation of 10 measurements of blank divided by the slope of calibration line, was 0.1 mg/L.

CONCLUSIONS

The objective of this investigation was to evaluate several commonly applied laboratory procedures that are used for plant-sample digestion in a closed-microwave system. The procedures were evaluated according to their effectiveness of analyte transfer into solution.

Normally, closed systems are considered to be very effective and free from analyte losses, but when the reaction is too vigorous, uncontrollable foaming occurs and might result in the loss of analytes. Loss of analytes also often occurs when samples are not completely digested and when digest solutions are evaporated completely. All these factors affect the accuracy and precision. Therefore, apart from the quantitative parameters of the evaluation, like calculated trueness and precision, observed completeness of digestion was also considered. The time between reagent addition and closure of the system appeared to be the most important factor in reducing the violence of reaction in the closed system and in improving the precision of digestion. However, application of this step strongly reduces the productivity of microwave digestion, especially when a single nest oven is used.

Comparison of the 10 digestion procedures applied to the tobacco digestion showed that the effectiveness of analyte transfer into solution differs significantly. The effectiveness also depends on the element considered. The largest difference was observed for calcium using procedures II and VIII. This difference could be related to the hydrofluoric acid application in the procedure II.

Two-way analysis of variance applied to procedures VII to X showed that the analyte transfer into solution was significantly dependent both on the digestion procedure and the element determined for tobacco, whereas for cabbage was depending only on the element. Nevertheless, they appeared to be effective, accurate and precise when applied to tobacco and cabbage reference material analysis. Although digestion of tobacco was still incomplete, the mean concentrations determined for subsequent elements were within the confidence intervals of the mean values of the reference materials. The procedure with the lowest total error for the tobacco elements determinations was procedure IX. Cabbage analysis using procedures VII and X produced the most precise and accurate results. The worst precision was for the determination of iron using procedure IX.

The residuals from the analysis of variance for tobacco, calculated using normalized data for the ‘internal quality control sample’ and reference material, were of the same order. For cabbage, the similar residuals for the ‘internal quality control sample’ were about half that for reference material. This could suggest that the materials were of similar homogeneity.

Because there were no data available on Si content in the reference materials examined, the results of our examinations cannot be directly considered for this factor. Although digestions were not complete for the tobacco samples, the determinations were satisfactorily accurate.

Our analysis did not cover all possible determinations, but the determination of chosen elements according to the described procedures allowed comparison of the main, minor, and trace plant component behaviour.

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