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COMPARISON OF TWO METHODS FOR DESTRUCTION OF BIOLOGICAL MATERIAL FOR DETERMINATION OF SELENIUM

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The purpose of the study was to compare two methods for destruction of biological material for selenium (Se) analysis: wet digestion by conductive heating in programmed digestion block and digestion in microwave oven. In both methods samples were prepared in a closed system using nitric acid. Selenium was analysed by electrothermal atomic absorption spectrometry. The results have shown that both methods are convenient for complete mineralisation and are accurate in determining selenium in a variety of foodstuffs. Microwave digestion, however, has the advantage of speed and simplicity over the conventional heating procedure.

Key words:
dry ashing, fish, meat, microwave digestion, wet digestion

Preparation of samples is a critical stage in the analysis of trace elements. The well-established standard methods usually involve wet digestion by conductive heating (hot plate, digestion block) or dry ashing (muffle furnace). Mineral acids, oxidants, and ashing aids are used in various sample preparation techniques (1–3). Two types of the wet digestion system are currently in use: closed-vessel and open-vessel system (1, 2). Ever since the first application of microwave energy as a heat source for wet digestion in 1975, (1) the method has increasingly been used for preparation of samples (1, 4, 5).

Some advantages of microwave digestion over conventional sample preparation techniques for volatile elements have already been described – short sample prepara-

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tion time, less acid required for digestion, pressure/temperature control, automation, and flexibility (1).

The purpose of our study was to compare and verify two methods for preparation of samples used in determination of selenium in food items: classical conductive heating in a programmed digestion block and digestion in a microwave oven. Electrothermal atomic absorption spectrometry (ET AAS) was used to quantify selenium.

METHODS

Equipment

For decomposition of samples we used a commercial microwave oven (CEM, USA, Model MDS-2000 with 950 W power) and a conductive heater with programmed digestion block (Tecator, Sweden, DS-40 and Autostep 1012 Controller). Digestion vessels (100 ml) for the microwave digestion consisted of a Teflon-PFA inner liner with a poly (ether imide) outer casing that can withstand a pressure of 1380 kPa (200 psi) and a temperature of 200 °C. For conductive heating in digestion block, we used borosilicate glass tubes (100 ml) with Teflon caps closed with special metal holders.

Varian AA300, Se Super lamp, pyrolytic coated partitioned tubes, and standard addition and deuterium background correction were used to measure selenium.

Reagents

We used nitric acid and selenium standard solution (1000±2 mg Se/L) of suprapur quality produced by Merck (Germany). The ascorbic acid and $PdCl_2$ as a matrix modifier were produced by Sigma (Switzerland) and Fluka (Germany), respectively. The conductivity of deionised water used in the experiment was 0.06 μS/cm.

Digestion procedures

For microwave digestion, 3 g of each fresh food item (hake, fried chicken, and a farm rainbow trout) or 0.2 g of lyophilised certified reference material (Community Bureau of Reference, Belgium – BCR, Pig kidney 186) were mixed with 3–5 ml of concentrated, suprapur HNO_3, and heated in the microwave oven. Tables 1 and 2 show detailed experimental conditions.

Table 1 *Experimental conditions for microwave oven digestion of fresh food items**

Parameter	Stage		
	I	II	III
Power (%)	70	70	70
Pressure (psi)	50	100	150
Running time (min)	10	10	5
Fan speed (%)	100	100	100

*3 g of fresh food was mixed with 5 ml of concentrated HNO_3.

Table 2 *Experimental conditions for microwave oven digestion of lyophilised certified reference material (BCR, Pig kidney 186)**

Parameter	Stage			
	I	II	III	IV
Power (%)	90	90	90	90
Pressure (psi)	20	40	80	135
Running time (min)	15	10	10	35
Fan speed (%)	100	100	100	100

*0.2 g of certified reference material was mixed with 3 ml of concentrated HNO_3.

For conventional heating in digestion block, 1 g of each of the above fresh food items or 0.5 g of lyophilised standard reference material were mixed with 1 ml of concentrated suprapur HNO_3. The samples were left to stand at room temperature in open tubes overnight. The tubes were then closed and heated in the block according to the temperature programme given in Table 3.

After digestion, both series of samples were adjusted to 10 ml with deionised water and analysed for Se by ET AAS.

Table 3 *Experimental conditions for conventional heating of fresh food items in digestion block or lyophilised certified reference material (BCR, Pig kidney 186) in a closed tube system**

Parameter	Stage		
	I	II	III
Temperature (°C)	50	60	80
Time increase (min)	10	10	10
Time at T (min)**	30	30	300

*1 g of fresh food item or 0.5 g of certified reference material was mixed with 1 ml concentrated HNO_3.
**Time at T indicates the duration of digestion at preset temperature

Electrothermal atomic absorption spectrometry

The ET AAS method was modified for Se measurements in food, as previously described for serum (6, 7). Before the measurement, Se (VI) was reduced to Se (IV) by mixing ascorbic acid and a matrix modifier ($PdCl_2$) with the sample. Standard addition method and deuterium background correction were applied for Se analysis in all samples of biological materials. The accuracy of both destruction techniques was tested by analysing pig kidney samples as certified reference standard.

RESULTS AND DISCUSSION

Conditions for microwave decomposition of lyophilised material differed from those for fresh food because of different mixture volumes taken in the decomposition tube

(Table 1 and 2). Higher power and longer decomposition time were needed for the lyophilised standard. At the end of decomposition procedure, all biological material was dissolved and the solutions were transparent. Decomposition by conventional heating did not require different temperature programmes. Measurements of the certified reference material showed that the recovery after destruction in the digestion block and in the microwave oven was 95.8% and 104%, respectively (Table 4). Such recovery is considered very good for both methods. The relative standard deviations, obtained by measuring ten replicates, were between 5.0% and 17.7%, depending on the concentration of Se in the tested biological material (Table 5). The precision was lower for Se concentrations in food below 0.1 μg/g.

Table 4 *Accuracy of the two methods for wet digestion*

Certified standard	Certified value (μg/g)	Determined value (μg/g)*	Recovery (%)	Digestion method
Pig kidney 186	10.3 ± 0.5	10.6 ± 0.4	104	Microwave oven
		9.9 ± 0.2	95.8	Digestion block

*$\bar{X}_{10}$±SD

Table 5 *Repeatability of Se measurements in biological material decomposed by the two methods*

Biological material	$\bar{X}_{10}$ ± SD (μg Se/g)	RSD (%)	Digestion method
Fried chicken	0.33 ± 0.02	5.8	Microwave oven
Hake	0.25 ± 0.02	6.0	Microwave oven
Hake	0.22 ± 0.01	5.0	Digestion block
Farm rainbow trout	0.08 ± 0.01	17.7	Digestion block

RSD – relative standard deviation

CONCLUSION

To conclude, both digestion methods can be used for a complete mineralisation and are accurate in determining selenium in a variety of food items. However, our study shows that microwave digestion has the advantage of speed and simplicity over the conventional heating digestion procedure.

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Sažetak

USPOREDBA DVIJU METODA RAZARANJA BIOLOŠKOGA MATERIJALA ZA MJERENJE SELENIJA

Priprema uzoraka kritična je faza određivanja elemenata u tragovima. Klasične metode razaranja biološkog materijala jesu mokro (digestijski blok) ili suho (mufolna peć) spaljivanje. Primjena mikrovalova u istu svrhu naglo raste tek osamdesetih godina.
U ovom radu uspoređene su dvije metode mokrog razaranja biološkog materijala za kasniju analizu selenija (Se): razaranje u aluminijskom bloku i razaranje mikrovalovima. Postupak kod obje metode proveden je u zatvorenim posudama koncentriranom HNO_3 suprapur čistoće. Obje metode validirane su određivanjem Se u standardnom referentnom materijalu istom metodom. Izmjereni rezultati uspoređeni su s referentnim vrijednostima. Točnost određena mjerenjem Se u referentnom materijalu spaljenom u bloku za digestiju iznosi 95,8%, odnosno 104% za spaljivanje u mikrovalnom uređaju. Relativna standardna devijacija (RSD), određena mjerenjem Se u 10 paralelno priređenih uzoraka, kretala se od 2,3 do 17,7% i od 3,8 do 6,0% ovisno o koncentraciji Se u ispitivanom materijalu. Može se zaključiti da su obje metode primjenjive za potpuno razaranje biološkog materijala za naknadnu analizu Se. Dakako, razaranje mikrovalovima ima prednost jer je sam postupak jednostavniji i brži.

Ključne riječi:
meso, mokro razaranje, razaranje mikrovalovima, riba, suho razaranje

Requests for reprints:

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