*Journal of Chromatography*, 371 (1986) 299–306
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 1057

# MICROWAVE EXTRACTION

# A NOVEL SAMPLE PREPARATION METHOD FOR CHROMATOGRAPHY

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## SUMMARY

The applicability of microwave irradiation to the extraction of various types of compounds from soil, seeds, foods and feeds as a novel sample preparation method for chromatography was investigated. Samples were ground and mixed with an appropriate solvent, methanol or methanol–water for polar compounds and hexane for non-polar compounds. The suspensions were irradiated for 30 s, but they were not allowed to boil. After cooling, the irradiation was repeated several times. The samples were then centrifuged, and aliquots of the supernatant were injected into a chromatographic column. The yields of the extracted compounds obtained by microwave irradiation were compared with those obtained by the traditional Soxhlet or shake-flask extraction methods. The microwave extraction method was more effective than the conventional methods. Due to the considerable savings in time and energy, this novel method is suitable for fast extractions of large sample series.

## INTRODUCTION

Chromatographic analyses are usually preceded by tedious sample preparation to extract the compounds to be analyzed from complex samples, *e.g.*, soil, plant materials, foods and feeds. Traditional extraction methods, Soxhlet, shake-flask, etc., are time and energy consuming. Due to the long extraction process, degradation of the components can occur.

The extraction of crude fat, antinutritives and organophosphate pesticides was investigated in this study. The microwave technique developed in our laboratory was compared with the traditional Soxhlet and shake-flask extraction methods. Crude fat determination is a tedious procedure that must be performed for several hours, as

0021-9673/86/$03.50 

described in the A.A.C.C. method[1], using hexane or light petroleum (b.p. 55–60°C) as solvents and the traditional Soxhlet equipment. In the case of the extraction of vicine [2,6-diamino-4,5-dihydroxypyrimidine 5-(D-glucopyranoside)] and convicine (2,4,5-trihydroxy-6-aminopyrimidine 5-(D-glucopyranoside)] from fava beans, an alcoholic medium[2] was preferred to acidic[3] or alkaline[4] solvents to avoid the degradation of the glucosides occurring at high or low pH[5]. During the traditional extraction of gossypol [1,1′,6,6′,7,7′-hexahydroxy-5,5′-bis(1-methylethyl)-3,3′-dimethyl-2,2′-binaphthalene-8,8′-dicarbaldehyde] from cottonseed[6], degradation of the extracted compound was observed. The published methods for the extraction of organophosphate pesticides give a good recovery but involve many steps[7,8].

The rapid microwave extraction method described is even suitable for the extraction of labile components from different complex matrices. Using microwave irradiation, the degradative effects of high temperatures can be avoided. The energy of the microwaves facilitates rapid desorption from the matrices.

## EXPERIMENTAL

### *Materials*

Yeast, lupine, maize, soya bean, "Robaby" (baby food made in Hungary), walnut, meat flour (cooked, dried animal protein concentrate used for feeding), fava bean and the soil samples were commercially available in Hungary. The cottonseed was a gift from Charles Rudd (Interconcepts Inc., Palo Alto, CA, U.S.A.). Methanol and hexane used for chromatography and for extractions were of HPLC grade (Merck, Darmstadt, F.R.G.). Phosphate salts and glacial acetic acid for the buffer solutions were of analytical grade (Reanal Fine Chemicals, Budapest, Hungary). Vicine and convicine standards were isolated by the procedure described by Brown and Roberts[9]. The gossypol standard was obtained from Sigma (St. Louis, MO, U.S.A.). The pesticide standards parathion [O,O′-diethyl O″-(*p*-nitrophenyl)thiophosphate] and bromophos [O,O′-dimethyl O-(2,5-dichloro-4-bromophenyl)thiophosphate] were obtained from Supelco (Bellefonte, PA, U.S.A.). Doubly distilled water was prepared in our laboratory using glass equipment.

### *Methods*

*Soxhlet extraction for antinutritives and crude fat.* Soya bean, cottonseed, walnut and fava bean samples were milled so as to pass through a 12-mesh (Tyler standard) sieve. A 3-g amount of each fava bean sample was extracted with 250 ml methanol–water (1:1, v/v) in a Soxhlet apparatus for 3 h to extract vicine and convicine. Each cottonseed sample (3 g) was extracted with 250 ml methanol–water (85:15, v/v) for 3 h for the gossypol determination. After the extraction, the solvents were evaporated under vacuum (for about 30 min) and the residue was dissolved in 5.0 ml of the extraction solvents.

The vicine, convicine and gossypol contents of the fava bean and cottonseed samples, respectively, were measured by high-performance liquid chromatography (HPLC).

The crude fat was Soxhlet extracted for 3 h using 250 ml hexane and 3 g of the yeast, lupine, maize, soya bean, cottonseed, "Robaby" , walnut and meat flour samples. After the extraction, the hexane solutions were evaporated under vacuum.

The weights of the dry residues were regarded as the contents of crude fat.

*Shake-flask extraction of pesticides from soil.* Soil samples were spiked with 0.05–0.25 mg/g parathion and 0.5–2.5 mg/g bromophos, respectively. The samples were incubated for 48 h at room temperature. A 25-g amount of each spiked samples was then suspended in 10 ml methanol and shaken in an erlenmeyer flask for 20 min. The extraction step was repeated three times with further 10-ml portions of methanol. The samples were centrifuged at 11 000 *g* and the pesticide content of 10-ml portions of the supernatant was evaluated by HPLC.

*Microwave extraction method.* The samples (0.5–1.0 g) were suspended in screw-cap vials with 2–3 ml solvent. The same solvents were used as those in the traditional extraction methods. The suspensions were irradiated for 30 s in a microwave oven, such as is commonly used in the kitchen (ER 638 ETD Type = 228, 1140 W, 2450 MHz; Toshiba, Tokyo, Japan). The suspensions were not allowed to boil; therefore, after 30 s they were cooled to room temperature for a few minutes; the irradiation step was repeated up to seven times in order to obtain the maximum yield of the extracted compounds. After irradiation, the samples were centrifuged at 11 000 *g* for 10 min, and aliquots of the supernatant were injected into the chromatographic column.

The solvents used for the extraction of the various compounds, the other sample preparation methods and the analytical methods used for the quantitation of the extracted compounds are summarized in Table I.

## *HPLC*

The HPLC analyses were carried out with a Liquopump Model 312 (LaborMIM, Budapest, Hungary) with Altex check-valves, equipped with a variable-wavelength UV detector (LaborMIM) and a Model 7010 sample injection valve (Rheodyne, Cotati, CA, U.S.A.) with a 20-$\mu$l loop. All separations were performed on a Dimesil $C_{18}$ (10-$\mu$m, 250 mm × 4.6 mm I.D.) column (Chromatronix, Mountain View, CA, U.S.A.). The chromatograms were recorded with an OH-814/1 recorder

TABLE I

SUMMARY OF THE TRADITIONAL EXTRACTION PROCEDURES

| *Origin* | *Compound extracted* | *Extraction solvent (conventional method)* | *Further sample preparation* | *Analytical method* |
|---|---|---|---|---|
| Food | Crude fat | *n*-Hexane (Soxhlet) | Evaporation to dryness | Weight measurement |
| Food | Vicine, convicine | Methanol–water (1:1, v/v) (Soxhlet) | Concentration in vacuum to 5 ml | HPLC |
| Food | Gossypol | Methanol–water (85:15, v/v) (Soxhlet) | Concentration in vacuum to 5 ml | HPLC |
| Soil | Pesticides | Methanol (shake-flask) | Centrifugation at 11 000 *g* for 10 min | HPLC |

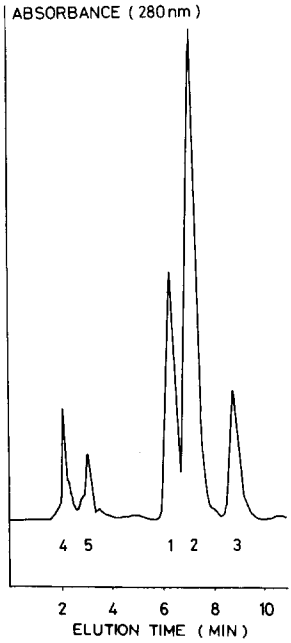
(Radelkisz, Budapest, Hungary) and integrated with an Apple II microcomputer.

The vicine and convicine contents of the fava bean samples were determined using 100% HPLC water as the mobile phase. The flow-rate was 1 ml/min and the detection wavelength 280 nm. The gossypol content of cottonseed was analyzed using water–methanol–acetic acid (12:85:3, v/v/v) as the mobile phase and a flow-rate of 2 ml/min. The detection wavelength was 254 nm. The separation of pesticides was carried out with 50 m*M* phosphate buffer, pH 2–methanol (85:15, v/v) as the mobile phase. The flow-rate was 1 ml/min and the detection wavelength 254 nm.

## RESULTS AND DISCUSSION

The extractions of polar (vicine, convicine, gossypol and pesticides) and non-polar (crude fat) compounds were carried out using the traditional (Soxhlet or shake-flask) methods and microwave methods. Typical chromatograms of vicine and convicine and the pesticides obtained after the microwave extraction are shown in Figs. 1 and 2.

The yield and the recovery of the extracted compounds obtained by the traditional methods were compared with those obtained by the microwave extraction



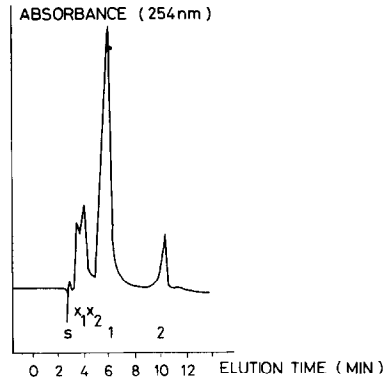




Fig. 1. Typical chromatogram of vicine and convicine obtained by microwave extraction from fava bean. Chromatographic conditions: mobile phase, neat water; flow-rate, 1.00 ml/min; column, Dimesil $C_{18}$ (250 mm × 4.6 mm I.D.); detection, UV at 280 nm. Peaks: 1 = convicine; 2 = vicine; 3 = internal standard (sulphaguanidine); 4, 5 = unknowns.

Fig. 2. Typical chromatogram of the pesticides obtained by microwave extraction from a spiked soil sample. Chromatographic conditions: mobile phase, 50 m*M* phosphate buffer, pH 2–methanol (85:15, v/v); flow-rate, 1 ml/min; column, as in Fig. 1; detection, UV at 254 nm. Peaks: s = solvent; $x_1$, $x_2$ = impurities; 1 = parathion; 2 = bromophos.

TABLE II

COMPARISON OF THE YIELDS OBTAINED BY THE TRADITIONAL AND THE MICROWAVE EXTRACTION METHODS

| *Origin* | *Compound detected* | *Concentration (% dry weight)* | |
|---|---|---|---|
| | | *Traditional* | *Microwave* |
| Yeast | Crude fat | 0.25 | 0.25 |
| Lupine (white) | | 7.4 | 6.2 |
| Maize | | 4.3 | 4.3 |
| Soya bean | | 16.3 | 15.5 |
| Cottonseed | | 28.5 | 26.7 |
| "Robaby" | | 22.8 | 21.9 |
| Walnut | | 67.9 | 65.6 |
| Meat flour | | 12.7 | 10.7 |
| *Fava beans* | | | |
| K-26 | Vicine, | 0.84 | 1.40 |
| K-1 | convicine | 0.70 | 1.07 |
| K-1/1 | | 0.63 | 0.98 |
| K-1/2 | | 0.60 | 0.80 |
| *Cottonseed* | | | |
| USA-1 | Gossypol | 0.69 | 2.00 |
| USA-2 | | 0.62 | 1.65 |
| SU-1 | | 0.49 | 1.44 |
| *Soil* | | *(mg)* | *(mg)* |
| 1 | Bromophos | 6.8 | 7.1 |
| | Parathion | 7.4 | 7.8 |
| 2 | Bromophos | 11.1 | 11.3 |
| | Parathion | 11.6 | 11.6 |
| 3 | Bromophos | 15.3 | 15.5 |
| | Parathion | 16.4 | 16.5 |

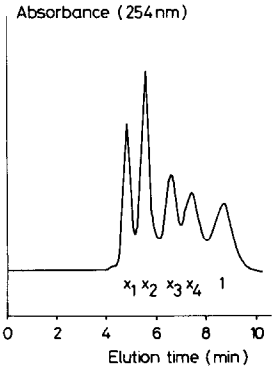



Fig. 3. Degradation of gossypol during the traditional Soxhlet extraction. Chromatographic conditions: mobile phase, water–methanol–acetic acid (12:85:3); flow-rate, 2.00 ml/min; column, as in Fig. 1; detection, UV at 254 nm. Peaks: 1 = gossypol; $x_1$–$x_4$ = breakdown products.

method (Table II). The data show that the microwave extraction method is more effective than the traditional Soxhlet extraction method in case of the polar compounds. As expected, the efficiency of the former was higher when the extraction solvents contained water. Since the dipole moment of water is high, the irradiation was more effective for the desorption and extraction processes of polar compounds.

The much higher recovery of gossypol from cottonseed obtained by the microwave irradiation can be explained by the fact that gossypol is sensitive to high temperatures, which are applied for hours during the Soxhlet extraction procedure. A chromatogram of the breakdown products of gossypol, produced during Soxhlet extraction, is shown in Fig. 3.

The yields of the crude fat extracted, with hexane, from various samples were almost the same for the traditional and the microwave extraction methods. However, the quantity extracted by microwave was slightly lower. The explanation for this could be that the molecular movement promoting the desorption processes caused by the microwave irradiation is much stronger in the presence of water or other solvents with high dipole moments.

To avoid the effect of high temperatures during the microwave extraction procedure, samples were irradiated for only 30 s at a time. They were then cooled to room temperature. The yield of the extracted compounds was investigated as a function of the number of irradiation steps. The results are shown in Figs. 4–6.

For the crude fat, five successive irradiation steps were needed for the maximum recovery (Fig. 4). For the maximum recovery of the polar compounds (vicine, convicine and gossypol), only two or three successive irradiation steps were needed. For gossypol, longer irradiation caused a slight decrease in the yield, due to degradation of the compound (Fig. 5). Fig. 6 shows the recovery of the organophosphate pesticides. In this case two steps were enough for maximum recovery.

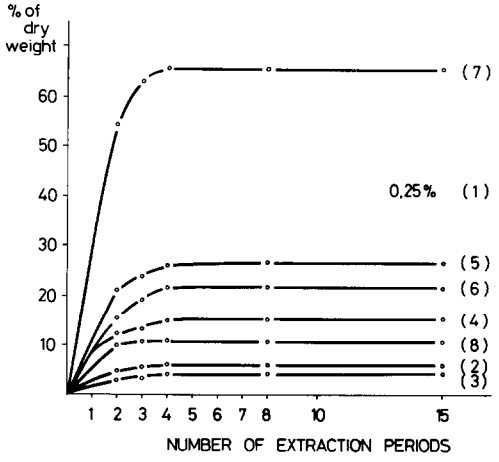



Fig. 4. The yield of crude fat as a function of the number of successive 30-s microwave irradiation steps. Curves: 1, yeast; 2, lupine (white); 3, maize; 4, soya bean; 5, cottonseed; 6, "Robaby"; 7, walnut; 8, meat flour.

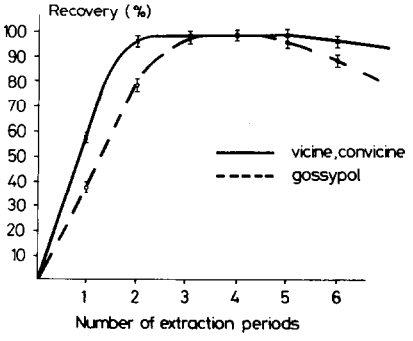



Fig. 5. Recovery of the antinutritives as a function of the number of microwave extraction steps (each 30 s). Vicine and convicine (——) were extracted from fava beans. Gossypol (– – –) was extracted from cottonseed.

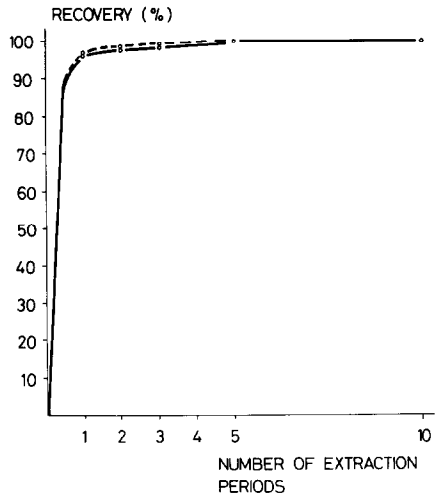



Fig. 6. Recovery of the organophosphate pesticides bromophos (——) and parathion (---) from soil as a function of the number of microwave extraction steps.

Table III summarizes the maximum efficiency and the time needed for the traditional and the microwave extractions of the compounds investigated. The times taken by the microwave extraction methods are almost 100 times less than those for the traditional methods. The microwave extraction method was more efficient than the Soxhlet extraction method in the case of polar compounds with water-containing solvents. Due to the shorter extraction time and the lower temperature, much higher recoveries were obtained for the sensitive gossypol molecule. The recovery of non-polar compounds by microwave irradiation was only slightly less with a non-polar water-free solvent.

TABLE III

COMPARISON OF THE RECOVERY OBTAINED AND THE TIME REQUIRED IN THE TRADITIONAL (a) AND THE MICROWAVE (b) EXTRACTION METHODS

$n$ = Number of determinations; C.V. = coefficient of variation.

| *Compound* | *n* | *Recovery (%)* | | *Time needed* | | *C.V. (%)* | |
|---|---|---|---|---|---|---|---|
| | | *a* | *b* | *a* | *b* | *a* | *b* |
| Crude fat | 17 | 100 | 98 | >3 h | <5 min | 0.5 | 0.03 |
| Antinutritives | 9 | 40–60 | 100* | ≥3 h | <5 min | High | 0.2 |
| Pesticides | 15 | 90 | 100 | <1.5 h | <1 min | 0.1 | 0.2 |

* Determined by use of spiked control samples.

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