Pollution extent in two therapeutical oil macerates of *Allium sativum* L.

STANCIC M., DOBRINAS S., BIRGHILA S.

Ovidius University,
124 Bvd. Mamaia, Constanta 8700; Romania

Summary

Two therapeutical garlic oil macerates (in olive oil and shark liver oil) were studied in order to establish the amounts of pollutants. A quantitative analysis of seven heavy metals (Cd, Cu, Fe, Mn, Ni, Pb, Zn) and nine organochlorine pesticides (lindan, HCB, DDT, DDE, DDD, heptachlor, aldrin, dieldrin, endrin) was carried out. The results of the experiments led to the conclusion that both oil macerates had low contents of Cd, Cu, Mn, Pb, Zn and some amounts of organochlorine pesticides (lindan, HCB, heptachlor, aldrin, dieldrin, endrin).

Key words: oil macerates, garlic in olive oil, garlic in shark liver oil, pollutant extent, heavy metals, flame atomic absorption spectrometry, organochlorine pesticides, gas chromatography coupled with electron capture detection

INTRODUCTION

Garlic, olive and shark liver oil have been used in health care for thousands of years. The olive oil garlic macerate contains vinyl dithiines, ajoene and allyl sulfides that lower the level of blood cholesterol, and have antiatherosclerosis and antifungical properties [1, 2, 3]. In addition, a macerate of garlic in fish liver oil is a powerful blood cholesterol lowering medicine because it contains eicosapentaenoic and docosahexan-enoic acids [4].

Environmental pollution is a risk for health. The two studied oil macerates are used to care of human health and for that reason it is important to know the amounts of pollutants they contain. Because of that the results of a quantitative analysis of the pollutant extent in the two oil macerates is presented in this paper. The contents of heavy metals were studied (because of their well-known high toxicity) [5]. In most biological samples the metals are usually present at low levels, therefore sensitive analytical techniques are required. Due to its relatively low cost and

excellent analytical performances, flame atomic absorption spectrometry (FAAS) was the technique used for analysing heavy metal contents in samples of the macerates. The amounts of organochlorine pesticides (POPs) were determined, too, because they belong to the most persistent organic pollutants and because of their toxicity and widespread use [6]. Gas chromatography coupled with electron capture detection (GC-ECD) was used for a POP assay.

MATERIALS

Samples

Garlic (*Allium sativum* L.) obtained in the local market; shark liver obtained from the Black Sea sharks (*Squalus achantias*); olive oil from the local market.

Materials

All metal stock solutions (1000 mg/l) were prepared by dissolving appropriate amounts of the metals or compounds in a dilute acid (1:1) and then diluting the obtained solutions with deionised water. The working solutions were prepared by diluting the stock solutions to appropriate volumes.

Pesticide standards (HCB, lindane, p,p'-DDT, p,p'-DDE, p,p'-DDD, aldrin, dieldrin, endrin, heptachlor) were supplied by International Atomic Energy Agency, Monaco laboratory. The usual sorbent material (Florisil: 60-100 mesh) was assayed for preconcentration step and was obtained from Fluka (packed in Switzerland). As eluents two organic solvents were assayed: n-hexane, supplied by Merck, Darmstadt, Germany, and dichlormethane supplied by J.T. Baker.

Sample preparation

For the preparation of shark liver oil, the technique proposed by Kates was used [7]. Frozen shark liver was broken up and heated in deionized water (on water bath). The oil layer on the surface was periodically collected. The collected oil was washed two, three times with warm deionised water in a separated funnel and then cooled to 2°C for the sedimentation of triglicerides. The trigliceride sediment was eliminated by filtration. The obtained filtrate (representing the shark liver oil) was conserved in brown bottles at dark and at 8-15°C.

For the preparation of oil macerate, fresh cloves of garlic were crushed and left for 10 minutes (so that biosynthesis of allicin from alliine and alliinase could take place). The oil (olive oil/shark liver oil) was added to fresh garlic (5 ml/g) and the mixture was shaken for 72 hours at room temperature. The obtained macerate was centrifuged at 6000 r/min for 10 minutes; the clear supernatant (representing the oil macerate) was retained and conserved at 5°C.

In order to determine the contents of heavy metals, each oil macerate of garlic was chemically mineralised with HNO_3 65% suprapur and H_2O_2 25% (2.5 ml of garlic oil macerate with 3 mL HNO_3 65% and 5 ml H_2O_2 25%) in a Digesdhal device provid-

Table 2 shows the retention times of POPs by GC-ECD. Detection limits (LOD) were calculated from spiked field blank chromatograms at the lowest concentrations, giving a response of three times the average of the baseline noise (3 injections). Gas chromatography peak identification was conducted by comparing gas chromatographic retention times with those of authentic standards.

Table 2

Quality parameters of the method.

no	organochlorine pesticide	retention time [min]	linear ranges [μg/l]	LOD [μg/l]
1	lindane	11.15	25-500	5
2	p,p'-DDT	16.98	25-500	10
3	p,p'-DDE	15.78	25-500	10
4	p,p'-DDD	16.22	25-500	10
5	HCB	10.53	5-500	4
6	aldrin	13.42	5-500	4
7	dieldrin	15.78	5-500	5
8	endrin	15.97	5-500	5
9	heptachlor	11.60	5-500	5

RESULTS AND DISCUSSIONS

Dynamics of heavy metal concentrations in oil macerates of garlic

Little is known about the localisation of heavy metals in garlic. Considering the compromise between low cost and required sensitivity, the flame-AAS method can be regarded as a suitable technique for determining Cd, Cu, Fe, Mn, Ni, Pb and Zn oil macerates of garlic [10]. The experimental data regarding the dynamics of heavy metals in the studied oil macerates is given in table 3.

Table 3

Dynamics of heavy metals and organochlorine pesticides in the studied oil macerates of garlic.

pollutant		concentration of the pollutant in the oil macerate	
		garlic in shark liver oil	garlic in olive oil
heavy metals (μg/l)	Cd	0.038 ± 0.0034	0.098 ± 0.0003
	Pb	0.020 ± 0.0005	0.030 ± 0.0015
	Cu	0.025 ± 0.0010	0.020 ± 0.0008
	Fe	28.152 ± 0.1602	20.845 ± 0.0069
	Mn	0.056 ± 0.0034	0.087 ± 0.0020
	Zn	1.304 ± 0.0014	0.260 ± 0.0007
	Ni	<LDL	<LD
organochlorine pesticides (μg/l)	lindan	0.158	0.138
	HCB	0.034	0.034
	DDT	-	-
	DDE	-	-
	DDD	-	-
	heptachlor	0.090	0.038
	aldrin	0.096	0.076
	dieldrin	0.012	0.044
	endrin	0.182	0.026

It is well known that Pb and Cd are major metallic pollutants. In spite of this, there are some acceptable maximum concentrations, according to international standards [11]; for example, 10 μg of Pb per 1 kg of soil and 0.5 μg of Cd per 1 kg of soil. As it can be seen in Table 3, Pb and Cd are present in both oil macerates, but their amounts do not represent a danger for health (0.020 μg Pb/l in the shark liver oil garlic macerate, 0.030 μg Pb/l in the olive oil macerate, 0.038 μg Cd/l garlic in the shark liver oil macerate, and 0.098 μg Cd/l garlic in the olive oil macerate).

Low concentrations of Cu, Fe, Mn, Ni and Zn are known to be necessary for normal development of vegetal and animal life; in high concentrations, however, they become toxic [11]. As it can be observed from table 3, Cu, Fe, Mn and Zn are present in the oil macerates in very low concentrations; therefore, their extent do not represent a danger for health.

Dynamics of POP concentration in oil macerates of garlic

The dynamics of POPs in the studied oil macerates is given in Table 3. ΣDDTs remains in the environment due to its resistance to degradation. The pesticide DDT is metabolised to DDE and DDD in the environment, but those compounds are slowly degraded under environmental conditions. Remarkably, DDT and its metabolites were not detected in the studied samples. The pesticide endrin was found to be present in the highest concentration of all the POPs (0.182 μg/l).

CONCLUSIONS

This paper presents original studies concerning analyses of contents of heavy metals and organochlorine pesticides (FAAS and GC-ECD, respectively) in two therapeutical oil macerates of garlic (garlic in shark liver oil and garlic in olive oil).

Both oil macerates have low contents of Cd, Cu, Mn, Pb, Zn and detected organochlorine pesticides (lindan, HCB, heptachlor, aldrin, dieldrin, and endrin).

REFERENCES

1. Koch HP, Lawson LD. Garlic: the science and therapeutic application of *Allium sativum L.* and related species. Baltimore: Williams and Wilkins, 1996:135-233.
2. Tapiero H, Townsend DM, Tew KD. Organosulfur compounds from *Alliaceae* in the prevention of human pathologies. Biomed Pharmacother 2004; 58(3):183-193.
3. Naganawa R, Iwata N, Ishikawa K, Kukuda H, Fujino T, Suzuki A. Inhibition of microbial growth by ajoene, a sulfur-containing compound derived from garlic. Appl Environ Microb 1996; 62(II):4238.
4. Adler AJ, Holub BJ. Effect of garlic and fish-oil supplementation on serum lipid and lipoprotein concentrations in hypercholesterolemic man. An J Clin Nutr 1997; 65:445-450.
5. Seiler HG, Sigel H. Handbook on toxicity of inorganic compounds. New York: Marcel Dekker, 1998:79.
6. Sherma J. Planar Chromatogr. Anal Chem 1991; 63:118-224.

7. Kates M. Laboratory Techniques in Biochemistry and Molecular Biology. Vol III. New York: Elsevier, 1986:204-209.
8. Chirila E, Carazeanu I, Bavaru A, Belc M. Determinarea metalelor grele din ecosistemul Marii Negre prin digestie. In: Proceedings of International Conference "Bramat 03"; 2003 Feb 24-26; Brasov, Romania; 2003:354.
9. Dobrinas S, Coatu V, Chitimiea CS. Utilizarea gaz cromatografiei pentru determinarea pesticidelor din apa de mare. Revista de Chimie 2002; 53:630.
10. Dobrinaş S, Matei N, Birghilă S, Popescu V, Soceanu A. Determination of heavy metals and C vitamin from *Allium* plants (garlic and onion). Environmental Engineering and Management Journal 2004; 3(4):689.
11. Ministry of Agriculture, Fisheries and Food. Standards of Food Surveillance Informartion Sheet. 1999; 131.

ZAWARTOŚĆ SUBSTANCJI TOKSYCZNYCH W DWÓCH LECZNICZYCH MACERATACH *ALLIUM SATIVUM* L.

STANCIC M., DOBRINAS S., BIRGHILA S.

Uniwersytet Ovidius,
124 Bvd. Mamaia, Constanta 8700; Rumunia

Streszczenie

Analizowano dwa wykorzystywane w celach leczniczych maceraty czosnku (w oliwie oraz oleju pozyskiwanym z wątrony rekina) w celu ustalenia zawartości substancji toksycznych. Przeprowadzono analizę ilościową zawartości siedmiu metali ciężkich (Cd, Cu, Fe, Mn, Ni, Pb, Zn) oraz dziewięciu pestycydów organochlorowych (lindanu, HCB, DDT, DDE, DDD, heptachloru, aldrinu, dieldrinu i endrinu). Ustalono doświadczalnie, że oba maceraty charakteryzują się niską zawartością Cd, Cu, Mn, Pb oraz Zn, a także wykrytych pestycydów organochlorowych (lindanu, HCB, heptachloru, aldrinu, dieldrinu i endrinu).

Słowa kluczowe: maceraty czosnku, oliwa, olej z wątroby rekina, substancje toksyczne, metale ciężkie, atomowa spektrometria absorpcyjna w wersji płomieniowej, pestycydy organochlorowe, chromatografia gazowa z detektorami wychwytu elektronów