♣Margarines, Butter and Vegetable Oils as Sources of Polycyclic Aromatic Hydrocarbons

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Concentrations of polycyclic aromatic hydrocarbons (PAH) were determined in Finnish butter, margarines and vegetable oils and their raw materials. In all the samples some degree of PAH contamination was found. The average per capita intake was estimated at 0.6 μg per day. The plant raw materials are supposed to be contaminated by combustion-derived atmospheric particles during the growing period. Inappropriate drying processes can substantially increase the contamination level. Deodorization processes used by food industry seem to decrease the total PAH levels significantly.

An essential part of the total human exposure to polycyclic aromatic hydrocarbons (PAH) is derived through food consumption. Calculations indicate the level of intake of PAH from food is 1.6–16 μg per day (1). PAH are present in grain and grain products; fruit; sugar and adjuncts; beverages; smoked fish and other aquatic foods; smoked, fried, grilled and roasted meat; and vegetable oils, margarines and fats (1).

Of the many existing studies on PAH in foods, only a limited number deal with vegetable oils and fats as sources of PAH (2–10).

Vegetable oils are a very heterogeneous group of foodstuffs consisting of a variety of raw materials processed in different ways. Some of the raw materials of the fat industry are dried while directly exposed to burning natural gas, or even to heating oil or burning wood, which almost certainly contaminates the material with PAH.

The amount of these contaminants is reduced in industry by subsequent treatment with active carbon or by steam distillation processes. Though the final concentrations of PAH in vegetable oils and fat products may be low, the high consumption of fats and oils makes them important sources of PAH. We collected common vegetable oils and fats in Finland to study their PAH contents, compare their PAH profiles and obtain information about the origin of the PAH present in oils and fats. Finally we estimated the intake of PAH from vegetable oils and fats in the Finnish diet. Some raw materials and samples from processes were studied as well.

EXPERIMENTAL METHODS

Samples 1–23 were chosen from the most consumed fat products on the Finnish market. Samples 24–26 were obtained from the margarine industry. The samples are listed in Table 1.

Forty g of sample was dissolved in 200 ml of cyclohexane and 50 ml methanol/water (4:1, v/v) was added. 3,6-Dimethylphenanthrene (25 μg/kg) was added

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as internal standard. The mixture was washed with 30 ml methanol/water (1:1, v/v) and with 50 ml water, after which PAH were extracted twice with 100 ml + 60 ml dimethylformamide/water 9:1 (v/v). Then 160 ml water was added and PAH were extracted with 100 ml + 60 ml cyclohexane. The extract was concentrated in vacuum to 1 ml.

The PAH concentrate was further purified and fractionated by SiO_2 column chromatography, 10 g SiO_2 (Kieselgel 60, Reinst 70–230 mesh) with 5% water content and 5 g Na_2SO_4 at the top of the column. The column was washed with 40 ml cyclohexane and the concentrate was added. PAH were eluted from the column with 150 ml cyclohexane. The cyclohexane solution was concentrated in vacuum to 75 μl. All the solvents were distilled.

The final concentrate was studied by gas chromatography/mass spectrometry (selected ion monitoring, SIM) with the aid of commercial reference compounds. The GLC column (25 m × 0.20 mm i.d. SE-54 fused silica) was temperature programmed from 70 to 275 C at 15 C per minute. The mass spectrometer was Hewlett-Packard 5970 A quadrupole EI MS operating with a resolution of about 1000. The monitored ions were as follows: m/e = 152 (biphenyl, acenaphtene, acenapthylene), 166 (fluorene), 168 (carbazole, methylbiphenyls, dibenzofuran), 178 (phenanthrene, anthracene), 179 (acridine), 184 (dibenzothiophene), 192 (2-methylphenanthrene, 2-methylanthracene,1-methylphenanthrene, 9-methylanthracene), 202 (fluoranthene, benzacenaphtylene, pyrene, 2-phenylnaphtalene), 206 (3,6-dimethylphenanthrene, 9,10-dimethylanthracene), 216 (benzo[a]fluorene, benzo[b]fluorene, methylpyrene), 226 (cyclopenta[c,d]pyrene, benzo[ghi]fluoranthene), 228 (benzo[a]anthracene, chrysene, triphenylene, naphtacene), 234 (retene, benzo[b]naphtho[2,1-d]thiophene),252 (1,1-binaphtyl, 2,2-binaphthyl, benzo[b-, j- and k]fluoranthenes, benzo[a]pyrene, benzo[e]pyrene, perylene, 3-methylcholanthrene), 254 (9-phenylanthracene, 6H-benzo[cd]pyren-6-one), 276 (indeno[1,2,3-cd]pyrene, benzo[ghi]perylene, anthanthrene), 278 (dibenzoanthracenes, benzo[b]chrysene), 300 (coronene), 302 (dibenzopyrene). The recoveries of the method were tested in several experiments and found to vary between 50 and 100% and to depend strongly on the molecular weights of the compounds (see Fig. 1). This dependency was presumably a reflection of differences in volatility. Because the use of one or two internal standards was clearly not sufficient, the results obtained by the internal standard method were corrected against the average recoveries of the individual compounds.

Using the injection volume of 2 μl and a splitless injection technique, the detection level (signal to noise = 3) of 10 pg of individual PAH on column was obtained. When 40 g of the sample was used and the final volume of the concentrate ws 75 μl, and assuming the average

TABLE 1

Concentrations of PAH in the Fat Samples in µg/kg

	1	2	3	4	5	6	7	8	9	10	11	12	13
Biphenyl	—	—	—	1.1	—	—	—	0.07	—	—	—	—	3.2
Acenaphtene	—	—	—	—	2.9	—	—	—	—	—	—	—	0.29
Acenaphtylene	—	—	—	—	—	—	—	—	—	—	—	—	n.a.
Fluorene	—	—	0.80	—	1.6	—	0.22	0.08	—	—	—	—	—
Methylbiphenyls	—	0.05	—	—	0.20	0.05	0.10	0.05	—	—	—	—	0.43
Dibenzofuran	—	0.23	—	—	0.64	0.13	0.15	0.08	—	—	—	—	0.36
Phenanthrene	0.72	0.56	6.0	1.1	5.1	0.47	0.69	1.1	0.69	0.29	0.45	0.20	2.1
Anthracene	—	—	0.92	0.31	0.29	0.36	—	0.04	—	0.25	—	—	0.16
Acridine	0.43	—	—	—	—	—	—	—	0.32	0.18	—	—	n.a.
Dibenzothiophene	0.05	0.03	0.40	—	0.46	0.08	0.03	0.05	—	—	0.05	—	0.23
2-Methylphenanthrene	0.19	0.10	1.3	0.23	0.96	0.26	0.23	0.85	0.94	—	0.68	—	n.a.
2-Methylanthracene	—	—	0.30	—	0.96	0.59	—	—	—	—	—	—	0.71
1-Methylphenanthrene	0.13	0.10	1.8	0.17	—	0.30	0.23	0.63	0.72	0.08	0.67	—	0.48
9-Methylanthracene	—	—	—	—	0.22	0.14	—	—	0.19	—	—	—	n.a.
Fluoranthene	—	1.4	9.0	—	0.52	—	1.5	—	—	—	—	—	1.1
Benzacenaphtylene	—	—	4.9	0.20	0.43	—	2.2	—	0.31	0.33	0.21	0.28	—
Pyrene	0.51	0.49	15	0.78	2.3	0.98	0.59	1.8	2.3	0.89	1.5	1.3	1.5
9,10-Dimethylanthracene	—	—	—	—	—	—	—	—	—	—	—	—	n.a.
Benzo(a)fluorene	—	0.03	3.8	0.07	0.37	0.19	0.10	0.55	0.53	0.28	0.44	0.42	0.71
Benzo(b)fluorene	—	—	2.1	0.03	0.24	0.11	0.10	0.35	0.53	—	0.51	0.29	0.51
1-Methylpyrene	—	0.03	2.9	0.10	0.34	0.23	0.16	0.43	0.67	0.25	0.48	0.38	n.a.
Benzo(b)naphto(2,1-d)thiophene	0.02	0.07	—	0.04	—	—	0.08	—	—	0.64	—	0.04	n.a.
Cyclopenta(cd)pyrene	0.03	0.13	—	1.1	0.59	—	0.10	—	—	—	—	—	n.a.
Benzo(ghi)fluoranthene	0.08	0.19	4.9	0.68	0.52	0.21	0.14	0.35	0.45	0.74	—	1.4	n.a.
Sum of benz(a)anthracene, chrysene and triphenylene	—	0.19	21	0.62	2.7	0.53	0.16	1.0	1.8	2.0	0.96	4.3	3.1
Naphtacene	—	—	—	0.19	—	0.10	—	0.07	—	0.31	—	0.62	n.a.
Retene	0.02	0.02	0.18	0.05	—	—	0.69	—	—	—	—	—	n.a.
Sum of benzo(b)-, -(j)- and -(k)-fluoranthenes	—	0.15	4.5	1.2	1.1	0.05	—	0.05	0.57	1.1	0.20	2.6	5.0
Benzo(e)pyrene	—	—	1.8	0.58	0.48	—	—	—	0.31	0.58	0.09	1.2	1.4
Benzo(a)pyrene	—	—	2.2	0.97	0.56	—	—	—	0.22	0.73	0.05	1.7	1.1
Perylene	0.02	—	0.57	0.28	0.21	—	—	—	0.10	0.13	0.02	0.48	0.11
Indeno(1,2,3-cd)pyrene	0.03	0.07	0.73	1.1	0.28	0.03	0.03	—	0.18	0.32	0.03	0.70	n.a.
Dibenzo(a,c)- and -(a,h)-anthracenes	—	—	—	0.08	0.07	—	—	—	—	—	—	—	0.06
Benzo(k)chrysene	—	—	0.16	—	—	—	—	—	—	—	—	—	n.a.
Benzo(ghi)perylene	0.05	0.08	0.66	1.4	0.22	0.04	0.02	—	0.22	0.34	0.07	0.69	1.1
Anthanthrene	—	0.02	0.09	0.53	0.03	—	—	—	—	0.08	—	0.10	n.a.
Coronene	—	—	0.23	—	—	—	—	—	—	—	—	—	n.a.
1,2,3,4-Dibenzopyrene	—	—	—	—	—	—	—	—	—	—	—	—	n.a.
Total PAH	2.4	4.0	86	13	24	4.8	7.5	7.6	11	9.6	6.4	17	23

TABLE 1 (*Cont.*)

	14	15	16	17	18	19	20	21	22	23	24	25
Biphenyl	1.4	—	—	—	—	0.07	—	—	—	—	97	0.65
Acenaphtene	—	—	—	—	1.43	0.22	—	—	1.1	—	45	0.50
Acenaphtylene	—	—	—	—	—	0.07	—	—	—	—	n.a.	n.a.
Fluorene	—	—	0.41	—	—	—	—	—	—	0.11	200	1.1
Methylbiphenyls	0.41	—	0.18	—	—	0.13	0.61	—	—	0.03	18	0.15
Dibenzofuran	0.61	—	—	—	—	0.89	—	1.9	—	0.03	520	1.1
Phenanthrene	1.5	0.09	5.0	0.14	0.58	3.2	4.7	4.0	0.47	8.5	1400	8.7
Anthracene	0.20	0.22	—	—	—	0.29	—	—	—	1.3	460	1.1
Acridine	—	—	0.89	0.05	—	—	—	—	—	0.25	n.a.	n.a.
Dibenzothiophene	—	0.02	0.86	0.03	0.03	0.51	0.76	0.49	—	0.08	74	0.55
2-Methylphenanthrene	—	0.03	1.6	0.07	0.37	0.36	1.0	1.9	1.7	3.2	n.a.	n.a.
2-Methylanthracene	0.59	0.07	1.8	—	0.25	—	—	—	0.97	1.5	270	3.5
1-Methylphenanthrene	0.41	—	—	—	—	0.67	0.86	—	—	4.3	190	5.0
9-Methylanthracene	—	0.27	—	—	—	0.30	—	—	—	0.97	n.a.	n.a.
Fluoranthene	1.6	1.4	0.84	—	—	—	0.40	4.0	0.70	18	460	23
Benzacenaphtylene	—	0.30	—	—	—	0.23	0.39	—	0.66	—	n.a.	n.a.
Pyrene	2.3	0.04	3.1	—	1.3	1.1	2.1	8.5	3.2	20	330	29
9,10-Dimethylanthracene	—	—	—	—	—	—	—	—	—	—	n.a.	n.a.
Benzo(a)fluorene	1.2	0.02	0.56	—	0.43	—	0.44	1.4	1.2	4.2	130	7.3
Benzo(b)fluorene	0.71	—	0.34	—	0.22	—	0.19	1.3	1.2	2.3	45	2.7
1-Methylpyrene	—	0.02	3.0	—	0.32	—	0.22	1.3	1.3	3.0	n.a.	n.a.
Benzo(b)naphto(2,1-d)thiophene	—	0.04	—	—	—	—	—	—	—	—	n.a.	n.a.
Cyclopenta(cd)pyrene	—	0.09	—	—	—	—	—	—	—	1.4	n.a.	n.a.
Benzo(ghi)fluoranthene	—	0.05	0.36	—	0.76	—	0.51	1.3	0.96	2.4	n.a.	n.a.
Sum of benz(a)anthracene, chrysene and triphenylene	5.3	0.04	1.5	—	3.5	0.41	2.8	5.7	5.9	3.3	190	5.7
Naphtacene	—	—	—	0.07	0.78	0.11	—	0.15	1.3	1.0	n.a.	n.a.
Retene	—	0.05	—	—	—	—	—	—	—	—	n.a.	n.a.
Sum of benzo(b)-, -(j)- and -(k)fluoranthenes	7.2	—	0.41	0.09	2.5	0.66	—	1.3	2.4	0.15	91	1.0
Benzo(e)pyrene	2.1	0.05	0.21	0.04	1.5	0.43	—	0.80	1.4	0.06	23	0.4
Benzo(a)pyrene	1.9	—	0.16	0.02	1.2	0.42	—	0.74	1.3	0.02	24	0.2
Perylene	0.08	—	0.08	0.09	0.58	0.92	—	—	0.55	—	5.9	—
Indeno(1,2,3-cd)pyrene	—	—	—	—	0.85	0.21	—	0.30	—	0.02	n.a.	n.a.
Dibenzo(a,c)- and -(a,h)-anthracenes	0.30	—	—	0.03	0.12	—	0.04	—	—	—	1.1	—
Benzo(k)chrysene	—	—	—	—	—	—	—	—	0.13	—	n.a.	n.a.
Benzo(ghi)perylene	0.99	—	0.12	0.10	0.88	0.20	0.58	0.34	0.82	0.02	10	0.0
Anthanthrene	—	—	—	—	0.18	0.04	0.29	—	—	—	n.a.	n.a.
Coronene	—	—	—	—	—	—	—	—	—	—	n.a.	n.a.
1,2,3,4-Dibenzopyrene	—	—	—	—	—	—	—	—	—	—	n.a.	n.a.
Total PAH	29	2.8	21	0.71	18	11	16	36	27	76	4600	92

[a]1. Butter; 2. Butter and vegetable oil mixture (80% butter); 3-6. Cooking margarines; 7-14. Table margarines; 15. Sunflower oil; 16. Cold pressed sunflower oil; 17. Corn oil; 18. Cold pressed corn oil; 19. Rapeseed and turnip rapeseed oil; 20. Olive oil; 21,22. Soybean oil; 23. Coconut fat; 24. Crude coconut oil; 25. Deodorized coconut oil. n.a., not analyzed; —, not detected (< 0.02 μg/kg).
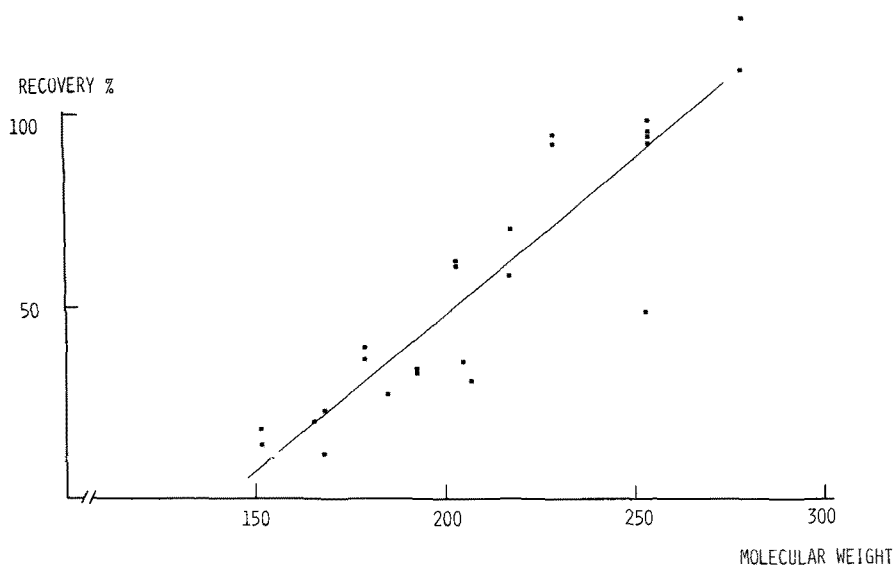
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FIG. 1. Recovery of the method as a function of the molecular weight of PAH compounds.

TABLE 2

Raw Materials Used in the Finnish Margarine Industry in 1983

Material	Percentage
Domestic	
Rapeseed and turnip rapeseed oil	21.7
Carcass fat	25.5
Milk fat	0.4
Imported	
Soy and sunflower oil	32.7
Other vegetable oil (coconut, sunflowerseed)	12.3
Fish oil	7.4
Total	100

recovery to be 50%, a detection level of 0.02 μg of PAH in kg sample was obtained. Disturbing components in the sample may occasionally lower the detection level. The metho was tested by making five replicate determinations of fluoranthene at the level of 1 μg/kg. The standard deviation was 15%.

Blanks were studied in connection with every series of samples (4–6 samples).

RESULTS AND DISCUSSION

The PAH contents in the samples studied are listed in Table 1. The yearly per capita consumption of fats and oils in Finland in 1982 was 7.2 kg (6.2 kg on fat basis) margarines, 11.3 kg (9.2 kg) butter, 0.8 kg mixtures of butter and vegetable oils, 1.8 kg vegetable oils and 1.3 kg carcass fat. Thus the total yearly intake (on fat basis) was 19.3 kg and intake 55 g per person. In addition to this, 24.3 kg fat is consumed each year in milk, cheese, meat, egges, fish and vegetables.

The average PAH content was 2.4 μg/kg in butter, 4.0

μg/kg in butter-vegetable oil mixtures (80% butter), 32 μg/kg in baking and frying margarines, 12 μg/kg in soft margarines and 23 μg/kg in vegetable oils. The results varied widely from product to product.

Calculated on the basis of the average consumption of fats and their PAH content, the yearly intake of PAH compounds is about 27 μg from butter, 160 μg from margarines, 40 μg from vegetable oils and 3 μg from butter-vegetable oilsmixtures, for a total of 230 μg yearly and 0.6 μg daily per person.

With the total intake of PAH compounds from food estimated at 1.6–16 μg per day (1) and more specifically at 3.7 μg per day in one study (2), fats must be considered an essential source of PAH compounds in the diet.

The basis of calculation of PAH concentrations varies form study to study and the results must therefore be compared with caution. Crude vegetable oils have been found rich in PAH compounds; coconut oil and copra in particular contain large amounts of PAH compounds (4,6–10). Table 2 lists the raw materials used in teh Finnish margarine industry.

Table 1 shows from the high value for the crude copra that it was liable to smoke during the drying process.

Deodorization by steam distillation seems to be very effective in removing PAH compounds. The concentration of PAH in the deodorized oil was about 1/10 that in the raw material (Table 1). The profile of the deodorized oil bears a resemblance to that of the raw material, but the proportional concentration of the compounds of low mol wt has decreased, as expected.

No information was available on the possible use of active carbon in purifying the fats in the products analyzed.

The profiles of the PAH species with reported carcinogenic activity (12), e.g., benzo(b)-, (j)- and (k)fluoranthenes, benzo(a)pyrene, benzo(ghi)perylene, indeno(1,2,3-cd)pyrene and dibenzoanthracenes, are similar in most samples. Therefore, the total PAH figures can be used in estimating the possible carcinogenic risk of individual samples.

Cyclopenta(cd)pyrene and benzo(b)naphtho(2,1-d)thiophene are considered indicators of contamination from traffic emissions. these compounds were found in varying concentrations in most of the samples studied.

Apparently the main source of PAH contamination of fats and oils is man-induced combustion. The atmospheric fallout of adsorbed PAH compounds is deposited on the plants and other raw materials used in fat production and the contaminants are partly transferred to the food products. As mentioned above, drying processes in which large volumes of hot ambient air are blown through the seeds may essentially raise the contamination level.

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♣Isolation of Lipase from Germinating Oilseeds for Biotechnological Processes

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Germinating oilseeds have been explored as a possible source of lipases (glycerol ester hydrolase, EC.3.1.1.3) for the biotechnological processing of oils and fats. Seedlings of rape (*Brassica napus*) and mustard (*Sinapis alba*) at day 4 of germination and cotyledons of lupine (*Lupinus albus*) seedlings at day 3 of germination yield active crude lipase preparations upon homogenization with Tricine buffer (pH 7.5) followed by centrifugation at 23,000 g. The major portion of the lipase activity, determined with an emulsion of sunflower oil as substrate, is recovered in the supernatant fraction. These crude lipase preparations exhibit highest activity between pH 8 and 9, but they are inactive in acidic pH or at pH > 10. Each of the crude lipase preparations is highly specific for the *sn*-1,3 positions of triacylglycerols. The crude lipase preparations exhibit excellent stability on storage at −10 C, but about 50-60% of their activity is lost upon freeze-drying. Dialysis of the crude lipase prior to freeze-drying does not prevent the loss of activity. However, acetone powder obtained from the seedlings exhibits a lipase activity as high as the undialyzed crude lipase preparation.

Seeds generally contain proteins and, depending on the plant species, mainly starch or triacylglycerols as food reserve for germination. In the mobilization of these three major reserves during germination, they are hydrolyzed initially by specific proteases, amylases and lipases, respectively.

Most investigations on plant lipases have been carried out on oleaginous seeds in which lipase activity is generally found to become prominent upon germination. During the germination of oilseeds the utilization of the storage fats is initiated by stepwise hydrolysis of the triacylglycerols to free fatty acids and glycerol. These primary reactions commonly are assumed to be catalyzed by the enzyme lipase (glycerol ester hydrolase, EC.3.1.1.3) which has been demonstrated to be active in seedling tissues of many different plant species (1).

Particularly in the endosperm of germinating castor bean seeds, lipolysis has been investigated in great detail (2-4). The castor bean lipase is localized in the membrane of the lipid bodies and is already active in ungerminated seed (5). This enzyme is rather unique among oilseeds, because most of the other oilseeds examined possess no detectable activity of lipase in extracts of ungerminated seeds (6).

Theimer and Rosnitschek (7) examined the development of lipase activity in the cotyledons of rape seedlings. They found that lipase activity reaches its maximum at day 4 and has its pH optimum at 9.0. Lin and Huang (8) studied the lipase in lipid bodies from the seeds of rape and mustard. They found that lipase activity is absent in the ungerminated seeds and increases during seedling growth. Lipase activity

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