Processing Effects on the Polyaromatic Hydrocarbon Content of Grapeseed Oil

S. Moret, A. Dudine, and L.S. Conte*

Dipartimento di Scienze degli Alimenti, Università degli Studi di Udine, 33100 Udine, Italy

ABSTRACT: Grapeseed oil can occasionally have dangerous levels for human health of polycyclic aromatic hydrocarbons (PAH) due to the drying process, which involves direct contact with combustion gases. Oil samples extracted from grapeseeds before and after drying were analyzed for their PAH content with a new, fast, bi-dimensional liquid chromatography method. Samples collected before drying had relatively high PAH amounts; Benzo(a)pyrene (BaP) content ranged from 0.9 to 2.4 ppb with an average of 1.4 ppb. The high contamination level found in the raw material is probably due to the practice of compacting pomace with bulldozers to reduce its volume before storage. The drying process did not significantly influence the light PAH content, but caused a large increase of the heavy fraction. BaP with an average content of 20.2 ppb, had the largest increase.

Paper no. J9314 in *JAOCS 77*, 1289–1292 (December 2000).

KEY WORDS: Bi-dimensional liquid chromatography, grapeseed oil, polycyclic aromatic hydrocarbons.

Polycyclic aromatic hydrocarbons (PAH) arise from incomplete combustion of organic matter. The optimal temperatures for their formation are in the range of 660–740°C [710°C for benzo(a)pyrene (BaP)] (1).

The ability to produce tumors in experimental animals after skin application, inhalation, intravenous injection and oral administration has been shown for various PAH, in particular BaP (1,2). Due to the multifactorial causes of cancer and the difficulty of extrapolating toxicity data from animals to humans, it has not been possible to date to establish PAH levels that constitute a health risk (2,3).

Some European countries (Germany, Austria, Poland) have adopted a legal limit of 1 ppb for BaP in smoked food. There are no legal limits for oils, but the German Society for Fat Science has fixed the following limits: 25 ppb for the sum of light and heavy PAH and 5 ppb for heavy PAH (including BaP) (4–6).

Fatty foods are particularly prone to PAH contamination because of their strong lipophilic characteristics. As food processes that involve drying and smoking can cause high PAH contents (5,6), special attention has to be devoted to grapeseed oil.

Grapeseed oil is characterized by a high content of linoleic acid (70–75%). Its composition makes it very desirable for inclusion in diets designed to lower serum cholesterol (7). Recovery of oil from grapeseeds involves drying the pomace (the solids remaining after grape pressing to obtain wine), separation of the seeds and extraction of the oil by pressing the seeds or by grinding the seeds and then extracting the oil with solvents (7). Crude grapeseed oil is then refined and dewaxed. PAH levels can be drastically reduced by refining, with the final level depending on the refining conditions (5).

Balenovic *et al.* (6) tested 36 grapeseed oil samples and 30 other vegetable oils for their PAH content. Light PAH concentrations (3 or 4 condensed rings) in the grapeseed oils were on average (127 μg/kg) twice the levels in the other oils while the heavy fraction (5 condensed rings or more) averaged (108 μg/kg) 15 times higher. They found exceptionally high BaP levels (20 μg/kg on average); similar results were obtained by Gertz and Kogelheide (5). The high amounts of PAH reported by these authors were detected in refined oils; no data were reported about oil processing and contamination level of the unprocessed grapeseed.

The object of this work was to evaluate PAH contamination in unprocessed grapeseeds and the increase in PAH due to the drying process carried out with rotary, direct-fired drum driers.

PAH were determined with a recently introduced (8) bi-dimensional liquid chromatography (LC) method, described in the Experimental Procedures section. It had good recovery and repeatability characteristics (8,9). One operator can analyze 10–12 samples in a day, reducing to a minimum sample manipulation and solvent consumption. Traditional methods, consisting of a saponification or a liquid–liquid partition step followed by column clean-up and high-performance LC (HPLC) determination (10–12), allow the analysis of only about four samples a day.

EXPERIMENTAL PROCEDURES

Reagents. To minimize sample contamination, all the solvents used for PAH extraction were distilled. The PAH standard mixture, 610 M (Superchrom, Milano, Italy) consisted of: acenaphtene (Ac), fluoranthene (Fl), naphthalene (Na), benz(a)anthracene (BaA), benzo(b)fluoranthene (BbF), BaP, benzo(k)fluoranthene (BkF), chrysene (Ch), acenaphtylene (Ap), anthracene (A), benzo(g,h,i)perilene (BghiP), fluorene (F), phenanthrene (Pa), dibenz(a,h)anthracene (DBahA), indeno(1,2,3-cd)pyrene (IP), and pyrene (P).

Samples. Pomace from different wineries was taken to a distillery producing grappa (an alcoholic beverage distilled from pomace) in the Italian Veneto region. The pomace was first

*To whom correspondence should be addressed at Dipartimento di Scienze degli Alimenti, Università degli Studi di Udine, Via Marangoni 97, 33100 Udine, Italy. E-mail: lanfranco.conte@dsa.uniud.it

amassed in the open, compacted with bulldozers and then stored in silos for a period varying from a minimum of 3 wk (time required for the fermentation of the residual sugar) to a maximum of 3 mon. After the distillation process, the exhausted pomace was dried in a direct gas-fired drum drier and the dried material was then sent to a vibrating screen in order to separate seeds from grape skins and stalks. The former were delivered to the oil extraction plant while the grape skins and stalks were recycled as combustible fuel.

Sampling was carried out after different storage times, each time collecting two samples: one before and one after drying. Samples were stored at −20°C before analysis.

Method. Fifteen grams of freeze-dried (model 1700 lyophilizer; Edwards Alto Vuoto, Milano, Italy) and ground (Moulinex grinder, Paris, France) grapeseeds was placed in a 300-mL flask with 150 mL of acetone and extracted in an ultrasonic bath (model 5200; Branson, Soest, The Netherlands) for 30 min. Freeze-drying facilitates grinding and avoids the dehydration step on anhydrous sodium sulfate. Acetone is a selective solvent for oil and PAH extraction and prevents the wax, which is present in grapeseed oil in high concentrations, from dissolving.

The extract obtained was vacuum-filtered through a 0.2 μm nylon filter (Chemtek, Bologna, Italy) and collected together with the washings (60 mL of acetone) in a round flask. The solvent was evaporated (T < 30°C) and the residue taken to constant weight by using a Rotavapor (model RE 120; Büchi, Flawil, Switzerland).

An aliquot (400 mg) of the fat was diluted with pentane in a 2-mL volumetric flask, and 400 μL of this solution was injected into a 250 × 4.6 mm i.d. silica column with a particle size of 5 μm (Spherisorb S5W; Waters, Milford, MA) using a mobile phase of pentane and 10% dichloromethane at a flow rate of 800 μL/min. The capacity of silica columns for retaining fat has been described (13,14). A syringe pump (Phoenix 30; Fison/CE Instruments, Milan, Italy) connected to a Varian (Palo Alto, CA) UV-vis model 9050 detector set at 254 nm was used.

As soon as the PAH fraction had been eluted, the column was backflushed with 10 mL of dichloromethane contained in a loop mounted on an additional valve (10 ports) and filled from a pressurized reservoir.

Light and heavy PAH fractions were collected separately in vials and evaporated to dryness under a stream of nitrogen. In order to identify the point of separation between the two fractions, all the samples were spiked with an amount of BaA able to produce a peak visible at 254 nm. Light PAH (from Na to P) eluted in the fraction between 5 and 7 min, while the heavy fraction (from BkF to IP) eluted between 7 and 10 min.

The analytical determination of PAH was carried out with a Varian model 9010 pump. The analytical column was a reversed phase C-18 column, 15 × 4.6 mm i.d. and a particle size of 5 μm (Supelcosil LC-PAH; Supelco, Bellefonte, PA) maintained at 35°C with a column heater (model L 7350; LaChrom, Merck, Darmstadt, Germany). The mobile phase consisted of water and acetonitrile at a flow rate of 1.5 mL/min. The light PAH residue from the silica column was diluted with 100 μL of acetonitrile and injected with a 20 μL loop, while the heavy residue was diluted with 70 μL of acetonitrile and injected with a 50 μL loop (in order to increase sensitivity).

TABLE 1
Wavelengths Selected for Light Polycyclic Aromatic Hydrocarbon (PAH) Detection

PAH[a]	λ excitation (nm)	λ emission (nm)
Na	275	330
Ac	275	330
F	275	330
Pa	250	365
A	250	402
Fl	240	470
P	240	385
BaA	270	390

[a]Abbreviations: Na, naphthalene; Ac, acenaphtene; F, fluorene; Pa, phenanthrene; A, anthracene; Fl, fluoranthene; P, pyrene; BaA, benz(a)anthracene.

The gradient elution program used for heavy PAH consisted of 50% water and 50% acetonitrile for 5 min, programmed to 90% acetonitrile over 15 min; for light PAH, the gradient was 5 min at 60% water and 40% acetonitrile going to 75% acetonitrile in 25 min and then to 100% acetonitrile in 1 min. PAH elution was monitored with both a Varian fluorometer model Fluorichrom (excitation filters: 7-54, 7-60; emission filters: 4-76, 3-73) and a Varian spectrofluorometer model 9070.

For the heavy PAH, the spectrofluorometer was used at a fixed excitation (290 nm) and emission (410 nm) wavelength, while for light PAH the excitation and emission wavelengths were programmed as reported in Table 1.

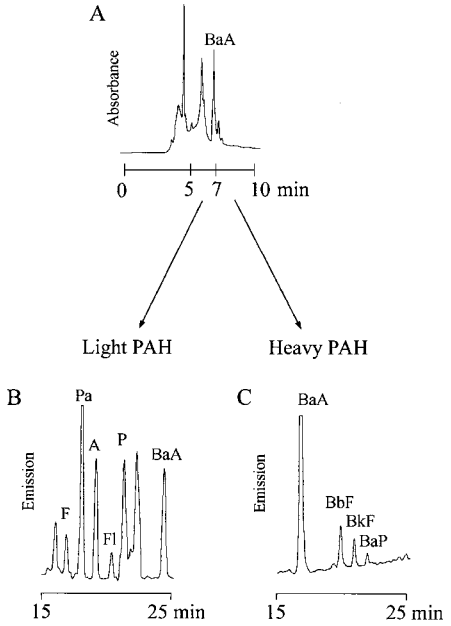

FIG. 1. (A) Ultraviolet trace obtained from the injection of the oil extracted from a grapeseed sample before drying into the silica column; (B) spectrofluorometric trace obtained, after reconcentration, from the injection of the light polycyclic aromatic hydrocarbon (PAH) fraction eluted from the silica column between 5 and 7 min into the C18 column; (C) fluorometric trace (C18 column) corresponding to the heavy PAH fraction eluted from the silica column between 7 and 10 min. Abbreviations: BaA, benz(a)anthracene; F, fluorene; Pa, phenanthrene; A, anthracene; Fl, fluoranthene; P, pyrene; BbF, benzo(b)fluoranthene;

TABLE 2
Heavy PAH Content (ppb) of Grapeseeds Before and After Drying[a]

Sample[a]	Storage (wk)	BbF		BkF		BaP		DBahA		BghiP		IP	
		Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
1	3	5.9	34.1	2.0	10.7	1.2	16.4	NQ	NQ	3.8	5.3	1.3	7.6
2	3	4.9	47.8	1.5	17.7	1.2	13.0	1.5	NQ	0.8	4.4	0.6	4.8
3	4	5.1	21.6	1.7	6.1	1.4	10.8	0.4	NQ	1.3	2.8	1.0	6.4
4	4	3.9	30.9	1.7	8.7	1.0	14.0	0.1	NQ	2.1	2.7	1.6	2.4
5	5	4.4	33.3	1.8	8.4	1.2	15.4	Trace	NQ	2.9	2.8	2.5	NQ
6	6	5.4	37.7	2.0	11.1	1.6	18.9	Trace	NQ	4.1	4.7	1.3	11.9
7	6	4.8	30.3	1.8	8.6	1.7	15.6	NQ	NQ	3.5	3.7	2.2	4.9
8	6	6.0	33.6	2.0	8.9	1.5	27.5	0.2	NQ	2.1	NQ	1.2	NQ
9	7	6.5	28.7	2.3	7.3	1.8	24.6	NQ	NQ	2.5	NQ	1.4	NQ
10	7	5.4	19.4	1.7	6.4	1.1	8.6	NQ	NQ	2.6	4.0	1.0	6.1
11	8	7.2	39.7	2.3	10.7	1.8	20.8	0.1	NQ	NQ	NQ	NQ	NQ
12	8	4.2	18.8	1.5	4.4	1.2	11.8	Trace	NQ	2.7	NQ	1.1	NQ
13	9	4.2	35.6	1.6	9.2	1.2	21.5	Trace	NQ	2.7	2.8	1.1	NQ
14	9	6.5	18.5	2.3	5.2	1.8	11.4	NQ	NQ	1.8	Trace	1.3	4.2
15	11	4.2	24.5	1.6	7.0	1.1	23.0	Trace	NQ	2.4	4.6	1.0	6.0
16	12	6.3	29.0	2.3	13.9	1.5	32.9	NQ	NQ	1.4	NQ	2.4	NQ
17	12	6.1	24.6	1.9	7.2	2.2	22.2	NQ	NQ	2.5	1.7	1.7	9.8
18	13	2.6	52.5	0.9	14.4	0.9	40.8	1.0	NQ	NQ	NQ	NQ	NQ
19	14	9.3	54.0	2.7	16.7	2.4	44.3	0.1	NQ	3.2	NQ	2.1	NQ
20	14	5.0	53.4	1.7	3.4	1.8	9.6	3.1	NQ	1.7	Trace	1.0	3.0

[a]All samples were dried by using methane plus grape skins and stalks as combustible, except sample No. 5 (only methane) and No. 11 (diesel fuels plus grape skins and stalks). Abbreviations: BbF, benzo(b)fluoranthene; BkF, benzo(k)fluoranthene; BaP, benzo(a)pyrene; DBahA, dibenz(a,h)anthracene; BghiP, benzo(g,h,i)perilene; IP, indeno(1,2,3-cd)pyrene; NQ, not quantified for the presence of interfering peaks; Trace, peak visible on the chromatogram, but not integrated. For other abbreviation see Table 1.

RESULTS AND DISCUSSION

The off-line LC–LC method recently introduced (8) for PAH determination in oils and lipid extracts and described here is well suited for rapid screening. Figure 1A shows the ultraviolet traces obtained from the injection of the oil extracted from a sample before drying into the silica column. Figures 1B and 1C show the light PAH spectrofluorometric trace (B) and the heavy PAH fluorometric trace (C) for the two corresponding fractions eluted from the silica column and injected, after reconcentration, into the reversed-phase column. It was necessary to collect the light and heavy PAH fractions separately because of the presence of interfering peaks. For light PAH detection it was possible to program excitation and emission wavelength changes (in order to maximize sensitivity for different PAH) as the traces were sufficiently "clean." For heavy PAH quantification, it was preferable to use data obtained with a fluorometric detector, which was able to give a "cleaner" trace (particularly in the case of samples after drying). Although the fluorometer is less sensitive than the spectrofluorometer, the high PAH contents found in grapeseed samples did not compromise their detection.

The heavy PAH chromatographic profile of samples after drying appeared to be complicated by the presence of some interfering peaks, which sometimes compromised correct quantification. A large "hump" under the peaks can also be observed, probably due to the presence of an incompletely resolved complex mixture of PAH isomers.

Data concerning heavy PAH, expressed as μg/kg of oil (ppb), are reported in Table 2, while Table 3 shows light PAH concentrations. Heavy PAH contents before drying appear very high if compared with those generally found in other raw vegetable oils. BbF was the most abundant PAH, with a level ranging from 2.6 to 9.3 ppb and an average content of 5.4 ppb. BaP content ranged from 0.9 to 2.4 ppb with an average value of 1.5 ppb. The high values can be explained considering that the pomace, amassed in the open, is compacted with motor vehicles (bulldozers) that could contaminate grapeseeds with exhaust gases or motor oil from the vehicles.

As can be seen from Tables 2 and 4 (reporting ratios of mean values for each PAH after and before drying), the drying process was always followed by a considerable increase in PAH content. This increase was particularly evident for BaP whose content, after drying, ranged from 8.6 to 44.3 ppb (average 20.2 ppb).

At the entrance of the rotating dryer, the pomace is directly exposed to gases at 650–750°C. PAH originating from incomplete combustion of organic matter (grape skins and stalks) can contaminate the seed surface.

All the analyzed samples except two were dried by using grape skins and stalks plus methane as fuel. Sample number 5 was dried with only methane whereas sample number 11 was dried with diesel fuel plus grape skins and stalks. We expected to find the sample dried with only methane less contaminated than the others, but the results showed no significant differences in relationship to the fuel used. It is important to consider that these results refer to a single sample obtained in such conditions that we cannot exclude sample contamination with the residue of organic matter (from previous processing) in the oven.

For each group considered (before and after drying), the PAH contents of the different samples were fairly homogeneous, and it appeared that storage time in silos did not influence PAH levels.

TABLE 3
Light PAH Content (ppb) of Grapeseed Samples Before and After Drying

Sample[a]	Storage (wk)	F		Pa		A		Fl		P	
		Before	After	Before	After	Before	After	Before	After	Before	After
1	3	12.9	27.5	129.7	223.7	13.6	28.3	85.6	92.5	107.2	193.2
5	5	17.3	22.5	158.5	173.4	15.9	18.3	109.2	112.0	172.7	159.0
6	6	12.0	15.7	128.1	110.6	12.1	12.7	n.q.	43.1	n.q.	84.6
10	7	34.1	30.1	132.9	129.1	7.5	10.1	70.4	58.6	79.4	65.6
14	9	27.3	33.6	157.4	210.1	10.2	16.0	92.0	79.4	93.4	110.1
15	11	19.2	33.2	124.8	263.1	8.8	16.6	90.6	74.8	115.4	103.9
17	12	13.8	8.9	132.6	130.6	9.7	10.4	116.5	58.8	199.1	n.q.
19	14	21.2	11.0	127.2	130.0	9.0	15.4	81.0	54.9	110.6	n.q.

[a]All samples were dried by using methane plus grape skins and stalks as combustible, except sample No. 5 (only methane) and No. 11 (diesel fuels plus grape skins and stalks). See Table 1 for abbreviations.

TABLE 4
Ratios Between Average PAH Contents (ppb) After and Before Drying Grapeseeds

PAH[a]	Before[a]	After[b]	Ratio
F	19.7	23.3	1.2
Pa	133.3	165.6	1.2
A	10.5	15.2	1.4
Fl	89.9	69.3	0.8
P	125.4	106.7	0.9
BbF	5.4	33.4	6.1
BkF	1.9	9.3	4.9
BaP	1.5	20.2	13.5
DBahA	0.8	NQ	—
BghiP	2.5	3.6	1.4
IP	1.4	6.1	4.4

[a]Mean PAH content (ppb) of grapeseed samples before drying.
[b]Mean PAH content (ppb) of grapeseed samples after drying. See Tables 1 and 2 for abbreviations.

In considering the homogeneity of heavy PAH content found in samples collected after different storage times, it was decided to analyze a smaller number of samples for light PAH. As can be seen from Table 3, all samples (both before and after drying) had a very high light PAH content. In both cases the most abundant PAH is Pa (with a mean content of 133.3 ppb before drying and 165.6 ppb after drying), followed by P and Fl. In contrast to the observations with heavy PAH, in this case no important increase in light PAH content after the drying process was recorded.

According to some authors (15), who reported the presence of large amounts of light PAH of metabolic origin in some vegetables, we cannot exclude that at least a small part of these compounds may be of natural origin.

ACKNOWLEDGMENT

This research was carried out within the framework of the Project "Innovazione Analitica per la Sostanze Grasse Alimentari Naturali e Transformate" with the Financial Support of the Italian Ministry of University (MURST).

REFERENCES

1. Bories, G., Tossicità degli idrocarburi policiclici aromatici e dei prodotti di pirolisi, in *Tossicologia e Sicurezza degli Alimenti*, edited by Tecniche Nuove, Milano, 1988, pp. 359–372.
2. Larsen, J.C., and E. Poulsen, in *Toxicological Aspects of Food*, edited by K. Miller, Elsevier Applied Science, London, 1987, pp. 205–212.
3. Zedeck, M.S., Polycyclic Aromatic Hydrocarbons, *J. Environ. Pathol. Toxicol. 3*:537–567 (1980).
4. Speer, K., E. Steeg, P. Horstmann, T. Kuhn, and A. Montag, Determination and Distribution of Polycyclic Aromatic Hydrocarbons in Native Vegetable Oils, Smoked Fish Products, Mussels and Oysters, and Bream from the River Elbe, *J. High Resolut. Chromatogr. 13*:104–111 (1990).
5. Gertz, C., and H. Kogelheide, Untersuchung und Beurteilung von PAK in Speisefetten und -ölen, *Fat Sci. Technol. 96*:175–180 (1994).
6. Balenovic, J., I. Petrovic, and M. Perkovac, Determination of Polycyclic Aromatic Hydrocarbons in Vegetable Oils, in *Proceeding of Euro Food Chemistry VIII*, Wien, September 18–20, 1995, Vol. 2, pp. 275–281.
7. Amerine M.A., R.E. Kunkee, C.S. Ough, V.L. Singleton, and A.D. Webb, in *The Technology of Wine Making*, edited by AVI Publishing Company Inc., Westport, Connecticut, 1980, p. 645.
8. Moret, S., and L.S. Conte, Off-line LC–LC Determination of PAHs in Edible Oils and Lipidic Extracts, *J. High Resolut. Chromatogr. 21*:253–258 (1998).
9. Moret, S., L.S. Conte, and D. Dean, Assessment of Polycyclic Aromatic Hydrocarbons Content of Smoked Fish by Means of a Fast HPLC/HPLC Method, *J. Agric. Food Chem. 47*:1367–1371 (1999).
10. Mariani, C., and E. Fedeli, Idrocarburi Policiclici Aromatici negli oli vegetali, *Riv. Ital. Sostanze Grasse 61*:305–315 (1984).
11. Guillen, M.D., Polycyclic Aromatic Compounds: Extraction and Determination in Food, *Food Addit. Contam. 11*:669–684 (1994).
12. Tamakawa, T.K., T. Kato, and M. Oba, Polycyclic Aromatic Hydrocarbons, in *Handbook of Food Analysis, Vol. 2: Residue and Other Food Component Analysis*, edited by L.M.L. Nollet, Marcel Dekker, Inc., New York, 1996, pp. 1641–1663.
13. Grob, K., I. Kaelin, and A. Artho, Coupled LC–GC: The Capacity of Silica Gel (HP)LC Columns for Retaining Fat, *J. High Resolut. Chromatogr. 14*:373–376 (1991).
14. Moret, S., K. Grob, and L.S. Conte, On-line High-Performance Liquid Chromatography–Solvent Evaporation–High-Performance Liquid Chromatography–Capillary Gas Chromatography–Flame Ionization Detection for the Analysis of Mineral Oil Polyaromatic Hydrocarbons in Fatty Foods, *J. Chromatogr. A 750*:361–368 (1996).
15. Ciusa, W., and A. Morgante, Gli Idrocarburi Policiclici Aromatici (IPA) come metaboliti naturali di vegetali e organi animali, *Riv. Merceol. 29*:5–65 (1990).

[Received July 12, 1999; accepted September 1, 2000]