

Stability of Polycyclic Aromatic Hydrocarbons during Heating

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ABSTRACT

The stability of three polycyclic aromatic hydrocarbon (PAH) standards, benzo[a]pyrene (BaP), benzo[a]anthracene (BaA) and dibenz[a,h]anthracene (DBahA) during heating was studied. The residual amount of each PAH was quantified by high-performance liquid chromatography (HPLC). Results showed that during heating in a closed system, the levels of BaA, BaP and DBahA in solid form or in hexane decreased both with increasing temperature and time. The degradation of each PAH during heating fits a first-order model, and the degradation rate constant (hr^{-1}) at 200°C was higher than at 100°C. Also, the PAHs in solid form possessed higher stability than in hexane. Either in solid form or in solution, BaA showed the highest degradation rate constant, followed by BaP and DBahA at 100°C. Similar result was found at 200°C, with the exception that BaA was more stable than BaP.

Key words: polycyclic aromatic hydrocarbon, heating, HPLC

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs), formed through incomplete combustion or pyrolysis of wood or gasoline, represent an important class of food carcinogens. More than 160 PAHs have been characterized in nature^(1,2), however, only 16 were selected as priority pollutants by the United States Environmental Protection Agency (EPA). Of the various PAHs, benzo[a]pyrene (BaP), benzo[a]anthracene (BaA) and dibenz[a,h]anthracene (DBahA) were reported to be the most mutagenic and carcinogenic^(2,3). However, the stability of these PAHs have been seldom investigated.

The effects of various processing methods, such as smoking^(4,5), grilling^(6,7) and roasting^(8,9), on the formation of PAHs have been well documented. Krofmacher *et al.*⁽¹⁰⁾ reported that the photodegradation of several PAHs, benzo[a]pyrene, pyrene and anthracene proceeded faster in solution than ash in the air. This result indicated that the stability of PAHs can be varied with the form it exists, under light storage. The objective of this study was to determine the stability of three PAH standards, BaA, BaP and DBahA, in solid form or in solution, during heating.

MATERIALS AND METHODS

I. Materials

Three PAH standards, including BaA, BaP and DBahA were purchased from Sigma Co. (St. Louis, MO, USA). Silicon oil was obtained from Merck Co. (Taipei, Taiwan). Ampoules were made from Pyrex glass according to a procedure by Chen and Meng⁽¹¹⁾. An HPLC polymeric C18 Envirosep-pp (125 × 4.6 mm I.D., 5 μm) was from

Phenomenex Co. (CA, USA). The HPLC-grade solvents such as hexane and acetonitrile were from Merck Co. (Darmstadt, Germany), and were filtered through a 0.2-μm membrane filter and were degassed by sonication prior to use.

II. Instrumentation

The HPLC instrument consists of two PU-980 pumps (Jasco Co., Tokyo, Japan), a UV/VIS 970/975 detector (Jasco Co.), an 821-FP fluorescence detector (Jasco Co.), and a Jasco MD-915 photodiode-array detector. A Borwin computer software (Le Fontanil, France) was used to process data.

III. Methods

(I) Heating of PAH Solution

A working solution of 50 ppm BaA or BaP was prepared by dissolving 5 mg of each in 100 mL hexane. Likewise, a working solution of 20 ppm DBahA was prepared by dissolving 2 mg in 100 mL hexane. A portion of each PAH solution (0.2 mL) was collected and transferred to an ampoule, which was sealed with a flame by oxygen/gas. The ampoule was placed in a thermostat-controlled oil bath, prefilled with 8 liters silicon oil as a heating medium. The temperature was preset at 100°C or 200°C. For 100°C, samples were collected at intervals of 0, 2, 5, 8, 12, 24, 36, 48, 60, 72, 84, 96 and 120 hr; for 200°C, samples were collected at 0, 20, 40, 60, 90, 120, 150, 180 and 240 min. All the ampoules were immediately inserted into ice water to terminate the reaction. The residual PAH solution was then transferred to a 2-mL vial and diluted to volume with hexane. The solution was filtered through a 0.2-μm membrane filter and then subjected to HPLC analysis. A total of 189 ampoules were used.

(II) Heating of Solid PAH

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Three working solutions of 50 ppm BaA, 50 ppm BaP and 20 ppm DBahA were each prepared as described above. A portion of PAH solution (0.2 mL) was collected and transferred to an ampoule. A stream of nitrogen was flushed into the ampoule for evaporation of solvent, after which the PAH was spread uniformly on the surface of the inner wall of the ampoule. The ampoule was then sealed with a flame by oxygen/gas. Likewise, the ampoule was heated at 100 and 200°C and samples were collected for HPLC analysis following a procedure shown above. A total of 189 ampoules were used.

IV. HPLC Analysis

An HPLC method similar to that described by Chen *et al.*⁽⁴⁾ was used to separate the various PAHs. Initially a mixture of acetonitrile/water (50:50, v/v) was maintained for 2 min, then linearly programmed to 100% acetonitrile within 23 min, and maintained for 15 min. The flow rate was 1.2 mL/min with UV wavelength at 287, 263 and 296 nm for detection of BaA, BaP and DBahA, respectively. Identification of each PAH was carried out by comparison of retention time and absorption spectra of unknown peaks with reference standards. Quantitation was made using an absolute calibration method. The calibration curve of each PAH was prepared by plotting six concentrations (1, 2, 3, 4, 5

and 10 ppm) against area. Triplicate analyses were conducted, and mean values were determined. The data were also subjected to analysis of variance and Duncan's multiple range test⁽¹²⁾. The detection limit of each PAH was determined based on a signal-to-noise ratio of 3 (S/N = 3).

V. Determination of the Rate Constant

The degradation of each PAH standard during heating was kinetically studied. The correlation coefficient (r^2) was calculated from the plot of the logarithm of the concentration of each PAH standard versus time. The degradation rate constant (hr^{-1}) was obtained from the following formula: $K = -\ln(CA/CA_0)/t$ where CA= the concentration of each PAH standard after heating, CA_0 = the initial concentration of each PAH standard, t = heating time.

RESULTS AND DISCUSSION

Figure 1 shows the absorption spectra of BaA, BaP and DBahA scanned at 200-400 nm. The maximum absorption wavelength of BaA, BaP and DBahA was found to be 287, 263, and 296 nm, respectively. To obtain the maximum sensitivity, these wavelengths were chosen for detection and quantification of PAHs. In most studies the simultaneous detection of 16 PAHs was often conducted by UV at 254nm (Chen *et al.*, 1996), which would make the quantitative data of the PAHs less accurate. For the reproducibility test, the coefficient of variation (CV %) of these three PAHs within days and between months were 0.52 and 0.71%, respectively. The detection limits of BaA, BaP, and DBahA were 0.38, 0.15, and 0.08 ng, respectively. Table 1 shows the linear equation and R square of the calibration curve of the PAHs. A linear correlation was observed between concentration and integrated area of each PAH, and the R square was more than 0.995 for all the three PAHs. Figure 2 shows the HPLC chro-

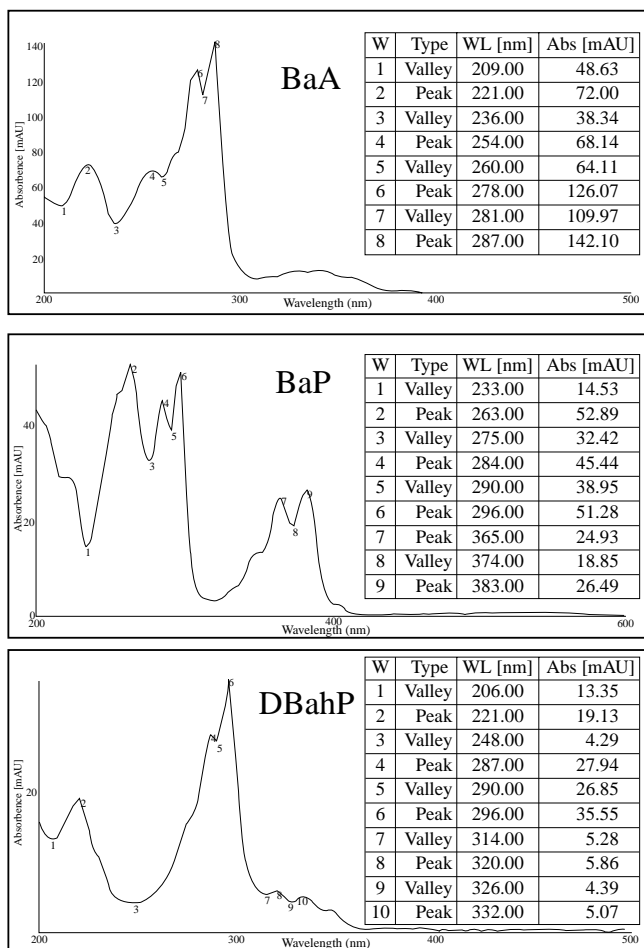


Figure 1. UV spectra of BaA, BaP and DBahA scanned at 200-400nm.

Table 1. Linear equations and R square of the calibration curves of BaA, BaP and DBahA

	Linear equation	R square
BaA	$y = 277825x - 93888$	0.9967
BaP	$y = 292718x + 65518$	0.9995
DBahA	$y = 1130728.9163x + 670496.9882$	0.9954

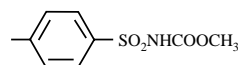


Figure 2. HPLC chromatogram of the blank reagent (n-hexane).

matogram of the blank reagent (hexane), demonstrating that no interfering peak or impurity was present. The recovery of BaA, BaP and DBaHA after heating and filtration through a 0.2 μm membrane filter was found to be $98.7 \pm 2.1\%$, $98.1 \pm 3.4\%$, and $99.1 \pm 1.3\%$ for triplicate analyses.

Figure 3 shows the HPLC chromatogram of the three PAHs heated at 100°C for 6 hr. No significant degradations were found for BaA or DBaHA after heating for 6 hr. However, a minor degradation of BaP occurred.

Nevertheless, this would not interfere with quantification of BaP. Table 2 shows the residual amount of BaA, BaP and DBaHA in solid form or in solution during heating at 100°C for 2-120 hr. The amount of each PAH, either in solid form or in solution, decreased with increasing heating time. Also, the loss of PAH in solution was found to be higher than that in solid form at 200°C. This is probably because that in a solid state, most PAH molecules were adsorbed on the surface of the ampoule glass, and thus caused a decrease of the mobili-

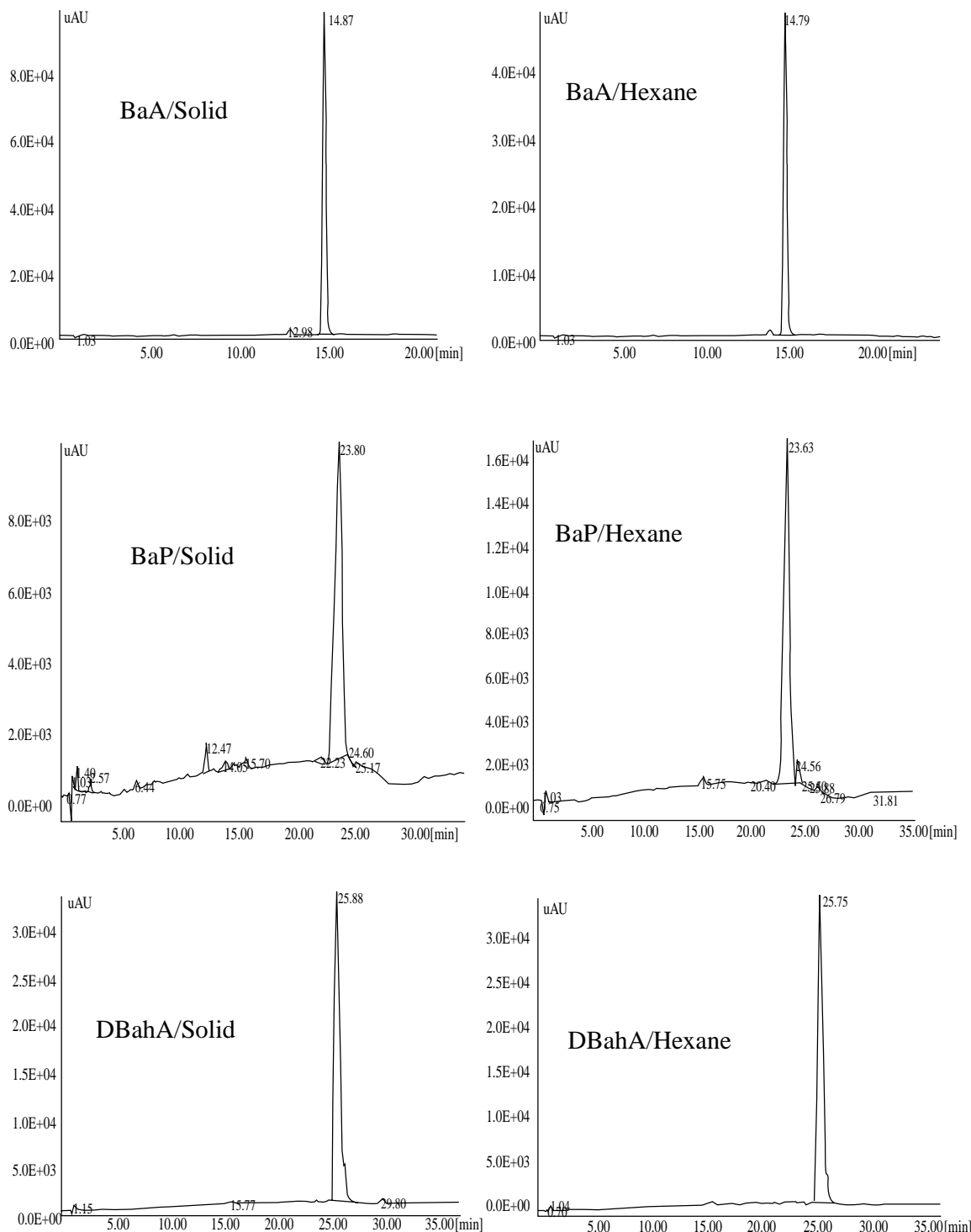


Figure 3. HPLC chromatograms of BaA, BaP and DBaHA in solid form and in hexane during heating at 100°C for 6 hr.

Table 2. Concentration (ppm) change of residual PAH during heating at 100°C

Time(hr)	Solid form			Liquid form		
	BaA ¹	BaP ¹	DBahA ¹	BaA ¹	BaP ¹	DBahA ¹
0	5.00 ± 0.04 ^a (0.00) ² (0.80%) ³	5.00 ± 0.02 ^a (0.00) (0.40%)	2.00 ± 0.01 ^a (0.00) (0.50%)	5.00 ± 0.02 ^{a4} (0.00) (0.40%)	5.00 ± 0.02 ^a (0.00) (0.40%)	2.00 ± 0.01 ^a (0.00) (0.50%)
2	4.87 ± 0.05 ^a (2.69) (1.03%)	4.88 ± 0.06 ^{ab} (2.36) (1.23%)	1.97 ± 0.03 ^a (1.56) (1.52%)	4.72 ± 0.09 ^b (5.62) (1.91%)	4.66 ± 0.04 ^b (6.89) (0.86%)	1.95 ± 0.01 ^{ab} (2.31) (0.51%)
5	4.45 ± 0.07 ^b (11.00) (1.57%)	4.74 ± 0.08 ^b (5.26) (1.69%)	1.92 ± 0.29 ^b (3.89) (15.1%)	4.30 ± 0.09 ^c (14.06) (2.09%)	4.14 ± 0.17 ^c (17.23) (4.11%)	1.88 ± 0.01 ^{bc} (5.78) (0.53%)
8	4.03 ± 0.05 ^c (19.31) (1.24%)	4.72 ± 0.91 ^b (5.68) (19.28%)	1.88 ± 0.02 ^c (6.22) (1.06%)	3.88 ± 0.14 ^d (22.50) (3.61%)	3.62 ± 0.27 ^d (27.57) (7.46%)	1.82 ± 0.03 ^{cd} (9.25) (1.65%)
12	3.70 ± 0.14 ^d (26.04) (3.78%)	4.52 ± 0.12 ^c (9.68) (2.65%)	1.81 ± 0.01 ^d (9.34) (0.55%)	3.31 ± 0.10 ^e (33.75) (3.02%)	2.93 ± 0.16 ^e (41.36) (5.46%)	1.76 ± 0.05 ^d (12.13) (2.84%)
24	3.10 ± 0.18 ^e (37.92) (5.81%)	4.31 ± 0.03 ^{cd} (13.87) (0.70%)	1.74 ± 0.04 ^e (13.00) (2.30%)	2.04 ± 0.70 ^f (59.22) (34.31%)	1.95 ± 0.16 ^f (60.96) (8.21%)	1.66 ± 0.03 ^e (17.20) (1.81%)
36	2.68 ± 0.04 ^f (46.48) (1.49%)	4.29 ± 0.06 ^d (14.19) (1.40%)	1.59 ± 0.03 ^f (20.71) (1.89%)	1.63 ± 0.06 ^g (67.36) (3.68%)	1.78 ± 0.71 ^f (64.45) (39.89%)	1.64 ± 0.05 ^e (17.80) (3.05%)
48	2.47 ± 0.11 ^g (50.65) (4.45%)	3.86 ± 0.02 ^e (22.70) (0.52%)	1.56 ± 0.01 ^f (22.14) (0.64%)	1.49 ± 0.06 ^g (70.26) (4.03%)	1.53 ± 0.07 ^g (69.41) (4.58%)	1.56 ± 0.02 ^f (21.90) (1.28%)
60	2.04 ± 0.08 ^h (59.16) (3.92%)	3.52 ± 0.07 ^f (29.54) (1.99%)	1.45 ± 0.01 ^g (27.72) (0.69%)	1.27 ± 0.23 ^h (74.57) (18.11%)	1.08 ± 0.13 ^h (78.35) (12.04%)	1.50 ± 0.06 ^{fg} (24.84) (4.00%)
72	1.95 ± 0.06 ^h (61.03) (3.08%)	3.38 ± 0.08 ^f (32.32) (2.37%)	1.44 ± 0.02 ^g (28.00) (1.39%)	0.83 ± 0.04 ⁱ (83.31) (4.82%)	1.01 ± 0.01 ^h (79.80) (0.99%)	1.45 ± 0.03 ^g (27.57) (2.07%)
84	1.72 ± 0.06 ⁱ (65.64) (3.49%)	2.93 ± 0.12 ^g (41.38) (4.10%)	1.42 ± 0.01 ^g (28.96) (0.70%)	ND ⁵	0.64 ± 0.09 ⁱ (87.19) (14.06%)	1.35 ± 0.04 ^h (32.46) (2.96%)
96	1.57 ± 0.17 ^{ij} (68.66) (10.83%)	2.87 ± 0.01 ^g (42.67) (0.35%)	1.28 ± 0.05 ^h (36.23) (3.91%)	ND	0.51 ± 0.10 ⁱ (89.76) (19.61%)	1.31 ± 0.10 ^h (34.35) (7.63%)
120	1.44 ± 0.08 ⁱ (71.20) (5.56%)	2.18 ± 0.22 ^h (56.45) (10.09%)	1.18 ± 0.03 ⁱ (40.89) (2.54%)	ND	0.47 ± 0.02 ⁱ (90.56) (4.26%)	1.28 ± 0.02 ^h (36.00) (1.56%)

¹ Mean of triplicate analyses ± standard deviation.

² Values in parentheses represent the degradation percentage of PAH.

³ Values in parentheses represent the coefficient of variation (CV%).

⁴ a-j symbols bearing different letters in the same column are significantly different (P < 0.05).

⁵ ND: not detected.

ty of PAH molecules. In contrast, when in solution, the PAH molecules possessed a higher mobility because of evaporation of hexane, which in turn resulted in a greater loss of PAH. Of these PAHs, BaA was the most susceptible to degradation when present in solid form during heating, followed by BaP and DBahA. However, when in solution, DBahA was the least susceptible to degradation, followed by BaA and BaP. Similar trend was found for the concentration change of BaA, BaP and DBahA in solid form or in solution during heating at 200°C for 20-240 min.(Table 3) The loss of each PAH, either in solid form or in solution, was higher at 200°C than at 100°C. For instance, during heating of the BaA solution at 200°C, a 50% reduction occurred after 2 hr heating. However, at 100°C, a 50% loss was not observed until heat-

ing time reached 20 hr.

In a similar study, Kamens *et al.*⁽¹³⁾ reported that the half life of PAH in wood soot at 20°C was about 30-60 min. However, in this study the half life of all the three PAHs at 100 or 200°C was longer than 1 hr, with the exception of liquid BaP at 200°C. The half life of each PAH was determined when the residual concentration equal to 50% of the initial concentration of PAH according to the linear equation of the logarithm of the concentration of each PAH standard versus time. This is probably because that in a closed system, less oxygen is available for thermal oxidation to proceed. Instead, the thermal degradation dominated during heating.

In this study the effect of light on PAH degradation was not researched, mainly because in preliminary experiments

Table 3. Concentration (ppm) change of residual PAH during heating at 200

Time(min) ¹	Solid form			Liquid form		
	BaA ¹	BaP ¹	DBahA ¹	BaA ¹	BaP ¹	DbahA ¹
0	5.00 ± 0.02 ^{ad} (0.00) ² (0.04%) ³	5.00 ± 0.02 ^a (0.00) (0.04%)	2.00 ± 0.01 ^a (0.00) (0.50%)	5.00 ± 0.03 ^a (0.00) (0.60%)	5.00 ± 0.03 ^a (0.00) (0.60%)	2.00 ± 0.02 ^a (0.00) (1.00%)
20	4.51 ± 0.01 ^b (9.79) (0.22%)	4.83 ± 0.07 ^b (3.35) (1.45%)	1.91 ± 0.03 ^{ab} (4.50) (1.57%)	4.47 ± 0.08 ^b (10.62) (1.79%)	2.46 ± 0.05 ^b (50.85) (2.03%)	1.76 ± 0.24 ^{ab} (12.00) (13.64%)
40	4.20 ± 0.10 ^c (15.92) (2.38%)	4.64 ± 0.02 ^c (7.28) (0.43%)	1.87 ± 0.01 ^{abc} (6.28) (0.54%)	3.64 ± 0.40 ^c (10.99%)	1.49 ± 0.05 ^c (70.26) (3.36%)	1.62 ± 0.22 ^{bc} (19.00) (13.58%)
60	4.03 ± 0.06 ^{cd} (19.34) (1.49%)	4.28 ± 0.02 ^d (14.33) (0.47%)	1.84 ± 0.02 ^{bc} (7.95) (1.09%)	3.22 ± 0.01 ^d (35.67) (0.31%)	1.30 ± 0.15 ^d (73.97) (11.54%)	1.54 ± 0.16 ^{bcd} (23.00) (10.39%)
90	3.98 ± 0.02 ^{de} (20.47) (0.50%)	4.27 ± 0.05 ^d (14.67) (1.17%)	1.82 ± 0.05 ^{bc} (9.16) (2.75%)	2.99 ± 0.06 ^e (40.23) (2.01%)	0.91 ± 0.02 ^e (81.83) (2.20%)	1.44 ± 0.06 ^{bcd} (28.00) (4.17%)
120	3.84 ± 0.17 ^e (23.28) (4.43%)	4.18 ± 0.02 ^d (16.42) (0.48%)	1.74 ± 0.04 ^{cd} (13.23) (2.30%)	2.11 ± 0.13 ^f (57.73) (6.16%)	0.50 ± 0.02 ^f (90.06) (4.00%)	1.30 ± 0.13 ^{cdef} (35.00) (10.00%)
150	3.54 ± 0.01 ^f (29.13) (0.28%)	3.89 ± 0.01 ^e (22.26) (0.26%)	1.61 ± 0.02 ^{de} (19.36) (1.24%)	2.00 ± 0.14 ^{fg} (59.93) (7.00%)	0.44 ± 0.02 ^f (91.14) (4.55%)	1.24 ± 0.06 ^{def} (38.00) (4.84%)
180	3.41 ± 0.02 ^f (31.83) (0.59%)	3.53 ± 0.05 ^f (29.34) (1.42%)	1.57 ± 0.03 ^e (21.75) (1.91%)	1.93 ± 0.06 ^g (61.33) (3.11%)	0.25 ± 0.03 ^g (94.90) (12.00%)	1.10 ± 0.30 ^{ef} (45.00) (27.27%)
240	2.98 ± 0.23 ^g (40.40) (7.72%)	3.04 ± 0.19 ^g (39.20) (6.25%)	1.42 ± 0.09 ^f (29.00) (6.34%)	1.44 ± 0.07 ^h (71.20) (4.86%)	0.18 ± 0.03 ^g (96.40) (16.67%)	1.00 ± 0.11 ^f (50.00) (11.00%)

¹ Mean of triplicate analyses ± standard deviation.

² Values in parentheses represent the degradation percentage of PAH.

³ Values in parentheses represent the coefficient of variation (CV%).³

⁴ a-h symbols bearing different letters in the same column are significantly different (P<0.05).

Table 4. Rate constant (hr⁻¹) of PAH degradation during heating at 100 and 200°C

Compound	100°C		200°C	
	Solid form	Liquid form	Solid form	Liquid form
BaA ^a	0.0108	0.0239	0.1139	0.3111
BaP ^a	0.0063	0.0206	0.1169	0.7649
DBahA ^a	0.0042	0.0037	0.0777	0.1669

^a Mean of triplicate analyses.

no significant concentration change of solid or liquid PAH was found when the ampoules were stored at room temperature under light for 24 hr. The data in Table 2 shows that the CV (%) for unheated solid or liquid PAH were all less than 1%, revealing that the effect of light on PAH degradation is insignificant. Likewise, this phenomenon indicated that in a closed system, less oxygen is available for the photooxidation to proceed. Kamens *et al.*⁽¹⁴⁾ studied the stability of PAH on wood soot particles and found no significant change of PAH occurred after absorption for 3-4 hr. A similar result was reported by Vaessen *et al.*⁽¹⁵⁾ when PAH was adsorbed on active carbon or glass surfaces.

Table 4 shows the degradation rate constant (hr⁻¹) of BaA, BaP and DBahA during heating at 100 or 200°C. The degradation of all the three PAHs during heating fit a first-order model because a linear correlation (r²>0.95) was found between the value of ln(CA/CA₀) versus time, where CA denotes concentration of each PAH after heating and CA₀

denotes the initial concentration of each PAH. Either in solid form or in solution, BaA showed the highest rate constant at 100°C, followed by BaP and DBahA. A similar result was found at 200°C, with the exception that BaA was more stable than BaP.

In most early studies the stability of PAHs was often conducted under oxygen and low temperature to monitor the oxidation. In this study, the three most toxic PAHs were selected to determine their stability in solid state or in hexane solution during heating. Two temperatures, 100 and 200°C, were chosen based on the reason that the former is frequently used for boiling while the latter for frying. Thus, the results of this study may provide basic information regarding the PAH removal during food processing, which is important to food safety.

From the results shown above it may be concluded that in a closed system, the degradation rate of each PAH was greatly dependent upon temperature and the form it exists.

Under the same temperature, the rate constant of each PAH in solution was greater than in solid form. Either in solution or in solid form, BaA showed the lowest stability at 100°C, followed by BaP and DBahA. However, at 200°C, BaP showed the lowest stability, followed by BaA and DBahA. Further research is necessary to determine the degradation products of each PAH during heating.

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多環芳香族碳氫化合物於加熱過程中之安定性

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摘 要

本研究以模式系統探討 benzo(a)pyrene (BaP), benzo(a)anthracene (BaA) 和 dibenzo(a,h)anthracene (DBaA) 三種多環芳香族碳氫化合物 (PAH) 以固態或溶液形態於加熱過程中之安定性，並以高效率液相層析法 (HPLC) 測定殘留 PAH 之含量。結果顯示，PAH 的熱裂解速率與加熱溫度及存在形態有直接相關。密閉狀況下，固態 PAH 含量或 PAH 溶液的濃度均隨著加熱溫度及時間增加而減少，三種 PAH 於 200°C 之熱裂解速率均遠大於 100°C，且其熱裂解均符合一次反應。在相同加熱溫度下，固態的 PAH 較 PAH 溶液安定。100°C 加熱時，BaA 具有最大的裂解速率數，其次為 BaP 和 DBaA；200°C 加熱所得到的結果與 100°C 類似，但 BaA 的安定性卻較 BaP 大。

關鍵詞：多環芳香族碳氫化合物，加熱，高效率液相層析法