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Decreased benzo(a)pyrene concentration in rapeseed oil packed in polyethylene terephtalate

Commercially available rapeseed oil, the same oil additionally refined physically, and paraffin oil for comparison purposes were spiked with benzo(a)pyrene (BaP) at the level of 29.4, 34.3, and 50.4 μg kg^{-1}, respectively, filled into polyethylene terephtalate (PET) cylindrical-shape receptacles, and the BaP concentrations were followed within 73 h by HPLC analysis. During this time, the BaP concentrations decreased to 22.9, 25.4, and 23.5 μg kg^{-1} due to sorption of BaP onto PET. Using a modified kinetic equation, diffusion coefficients of BaP in all oils were calculated. The values of the diffusion coefficients and distribution coefficients indicated that other compounds present in the oils competed with BaP and affected the rate of BaP diffusion in the oils and the extent of adsorption onto PET. Moreover, the curves of BaP concentration *vs.* time, obtained experimentally, exhibited oscillations in BaP concentrations around the adsorption curves calculated by the kinetic equation. This was observed especially at the initial stages of the experiments, with the subsequent establishing of steady equilibrium BaP concentrations between liquid and solid phases at the end of the experiments, which does not correspond with known theory of adsorption.

Keywords: Benzo(a)pyrene, polyethylene terephtalate, rapeseed oil, adsorption, kinetics, oscillation.

1 Introduction

Polycyclic aromatic hydrocarbons (PAH) include the largest class of known environmental carcinogenic compounds. Some of them, even though not carcinogenic, may act as synergists [1]. In a number of papers, remarkably high concentrations of PAH in fats and oils have been reported. Moret et al. [2] analysed 51 samples of olive oils and determined the total PAH concentrations from 2.94 to 143.12 μg kg^{-1}. Stijve and Hischenhuber [3] tested 12 samples of vegetable oils and determined the highest concentration of BaP in coconut oil (up to 581.7 μg kg^{-1}). Hopia et al. [4] found various PAH in Finnish butters, margarines and vegetable oils, and some raw vegetable oil materials, with total PAH concentrations in 25 samples varying from 0.17 (corn oil) to 4600 μg kg^{-1} (crude coconut oil). It was concluded that the enormous PAH concentrations in coconut oil could be brought about by direct drying of copra with smoke. With regard to the analytical findings as well as the harmful effect on human health, Slovak food legislation has limited the maximum acceptable concentrations of BaP in vegetable oil to the level of 5 μg kg^{-1} in 1995, and the International Olive Oil Council has recommended a value of 2 μg kg^{-1} as the maximum tolerable concentration of BaP in olive-pomace oil. At present, the European Union is going to amend Regulation (EC) No. 466/2001 to limit the maximum acceptable concentration of BaP in oils and fats intended for direct human consumption or for use as an ingredient in foods to the level of 2 μg kg^{-1}. When thinking about the interactions between foodstuff and packaging, the contamination of food by substances migrating from the polymer packaging is mostly intuitively considered. However, several papers have announced that the polymer packaging could also play an important detoxification role with regard to a high affinity of organic contaminants to some plastic materials. In this way, PAH concentrations were reduced effectively in a liquid smoke flavour by two orders of magnitude during 14 days of storage in polyethylene (PE) flasks [5]. As found, the rate-limiting step of the PAH sorption from liquid into PE is diffusion in liquid media [6]. As found later, PAH are primarily adsorbed on the PE surface, with subsequent diffusion into the polymer bulk [7]. Nowadays, the dominant plastic material used for vegetable oil and non-alcoholic drinks packaging is polyethylene terephtalate (PET). For packaging purposes, the food industry uses approximately 20% of the total world production, with an expected yearly increase by 15% in the near future. The ability of PET to decrease PAH concentrations in polar and non-polar liquid media has

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already been unambiguously proven [8]. However, the systems studied were too complicated to characterise more exactly the matrix effects on the rate and extent of PAH sorption. In order to eliminate the "each other" effects of PAH themselves in contact with PET, the behaviour of BaP as the only compound spiked into commercially available rapeseed oil was studied in this work. The results were compared with those obtained from the same but additionally physically refined oil and those obtained from paraffin oil as a totally inert non-polar oil matrix, free of any natural compounds.

2 Materials and methods

2.1 Materials

2.1.1 Rapeseed oil

Commercially available rapeseed oil, produced by Palma – Tumys Ltd. (Bratislava, Slovak Republic) was purchased in the local market. The oil was packed in a PET bottle with a volume of 4 L.

2.1.2 Physically refined rapeseed oil

A part of the rapeseed oil mentioned above was physically refined by molecular distillation at a lowered pressure of 10–20 Pa, with the temperature of the heating surface at 200 °C.

2.1.3 Paraffin oil

Paraffin oil was purchased from Merck, Germany.

2.1.4 PET receptacles

In the experiments, pre-bubbled PET receptacles of cylindrical shape with 2.2 cm i.d. were used. The receptacles were provided by Palma – Tumys Ltd. The company uses them for oil and fruit syrup packaging after blowing to a volume of 2 L.

2.1.5 BaP

BaP was of analytical grade, purchased from Supelco in a solid state. A solution for spiking was prepared by dissolving BaP in acetonitrile to the initial concentration of 500 mg L^{-1}.

2.1.6 Solvents

Acetonitrile was of gradient grade (Merck, Germany), methanol and hexane for analysis (Slavus, Slovak Republic). The solvents were rectified just before use in a distillation apparatus.

2.1.7 Other chemicals and materials

Anhydrous Na_2SO_4 and alumina were purchased from Merck, Germany.

2.2 Experiments

First of all, the oils were analysed for the presence of BaP. Subsequently, 100 g of the oil was spiked with BaP solution in a 2-L glass flask and left to spontaneously evaporate the solvent. To accelerate the evaporation, the oil was mixed occasionally. Then, roughly 900 g of oil was added, and the content of the flask was mixed thoroughly. At this stage, the sample of spiked oil was taken to determine the initial BaP concentration. Then, the receptacles were filled with the spiked oil and placed into a polystyrene box to protect them from light and maintain a constant temperature. The samples for analysis were taken after 1, 2, 3, 5, 7, 11, 14, 24, 49, and 73 h. To maintain the same static conditions and sampling during the experiments, a new couple of receptacles was taken for each analysis.

2.3 Sample preparation

Sample preparation was performed as by ISO 15302 as follows: 2 g of oil was weighed nearest 0.001 g into a 10-mL graduated flask, dissolved in hexane and diluted to the mark. Then, a chromatography column was filled with hexane, and 22 g alumina was immediately transferred to the column, and anhydrous sodium sulphate was added to the top of the column to form a layer about 30 mm thick. Then, hexane was left to fall to the level on top of the sodium sulphate layer, and 2 mL of the graduated flask content was applied onto the column. The column was eluted with hexane with a flow rate of about 1 mL min^{-1}, discarding the first 20 mL of eluate and then collecting 60 mL of eluate in a 100-mL round-bottom flask. The eluate was evaporated to about 0.5 mL and transferred into a mini-vial. Evaporation continued under nitrogen until near dryness. The round-bottom flask was rinsed twice with about 1 mL of hexane, which was transferred quantitatively to the mini-vial, and evaporation was continued under nitrogen. The evaporation was carried out just until dryness; the residue was dissolved in methanol and analyzed by HPLC.

2.4 HPLC analysis

The sample was applied onto a HPLC chromatograph consisting of a Rheodyne 7021 loop valve, a programming unit Pye Unicam 4003, a gradient pump Pye Unicam 4003, an integrator PU 4810 and a fluorescence detector with a programmable wavelength LC 1250 (GBC, Australia), which operated at 300 nm excitation and 410 nm emission wavelengths. Separation was carried out at ambient temperature on a LiChrolut column (25 cm × 0.4 cm i.d., packed with Lichrospher PAH, particle diameter 5 μm), and a LiChroCart pre-column (4 cm × 0.4 cm) was also used. The flow rate of the mobile phase was 1 mL min^{-1}. For determination, a combined isocratic and gradient elution was used as follows: binary gradient A – acetonitrile/water 50 : 50 (vol/vol), B – acetonitrile. Gradient: 3 min isocratic elution with A; then from 3 to 13 min B linearly from 0 to 53%; from 13 to 18 min isocratically; then from 18 to 23 min from 53% to 100% B; from 23 to 43 min isocratically; then return to initial conditions. All analyses were carried out in duplicate.

2.5 Determination of vitamins A and E

Oil (1–2 g) was weighed accurately into a flat-bottom flask with 1 g of ascorbic acid, 10 mL water, 35 mL pyrogallol ethanolic solution (0.5%) and 10 mL NaOH solution (60%, wt-%). The content of the flask was saponified under reflux for 30 min. After cooling, the content was diluted with 30 mL water and extracted four times with 10 mL hexane. The hexane extract was dried by filtration through anhydrous Na_2SO_4 and evaporated to dry matter using a rotary vacuum evaporator. The residue was dissolved in 5 mL methanol, micro-filtered and injected into a liquid chromatograph.

2.6 HPLC conditions of vitamin A and E determination

HPLC was performed isocratically on a Luna C18 analytical column (5 μm, length 25 cm, 4.6 mm i.d.) equipped with a security Guard C18 (40 × 3 mm, 5 μm). The temperature of the column was 28 °C; the mobile phase was methanol/water at a flow rate of 1.0 mL min^{-1}. The effluent from the column was directed to a UV detector operating at 290 nm.

2.7 Determination of BaP in paraffin oil

With regard to the paraffin matrix, neither the sample clean-up procedure nor HPLC determination was necessary. Therefore, the BaP concentrations were determined directly using a Genios fluorimetric reader (Tecan, Grödig bei Salzburg, Austria) equipped with an excitation filter with a pass maximum of 340 nm (bandwidth 10 nm) and an emission filter with a pass maximum of 460 nm (bandwidth 10 nm), using 10 flashes per measurement; integration time 40 s, gain adjustment and measurement from the bottom orientation. All measurements were carried out eight times.

3 Results and discussion

At first, the experiment was carried out with commercially available rapeseed oil. The experimentally obtained dependences of BaP concentrations *vs*. time were used for the calculation of the diffusion coefficient *D* using the kinetic equation (1) derived in [8], which is generally valid for non-stirred systems:

$$c_t = c_\infty + (c_0 - c_\infty) \sum_{n=1}^{\infty} \frac{4}{a^2\, \alpha_n^2} \exp\left[-D\, \alpha_n^2\, t\right] \quad (1)$$

D was calculated by the non-linear least squares method by minimizing the sum of squares of differences between the BaP concentrations measured experimentally and those calculated by eq. 1. BaP distribution between PET and oil expresses the distribution coefficient β:

$$\beta = \frac{c_0 - c_\infty}{c_\infty} \quad (2)$$

where c_0 is the initial BaP concentration in oil and c_∞ stands for the equilibrium BaP concentration in oil in infinite time, respectively. A higher value of the β coefficient corresponds to a greater decrease of BaP concentration in liquid media. As follows from Fig. 1, BaP concentration in the oil began to decrease immediately after filling the PET receptacles. Although BaP was the only PAH compound spiked, its diffusion coefficient had a value almost identical that obtained in the presence of five other PAH compounds [8], as shown in Tab. 1. On the other hand, the distribution coefficient β obtained in this experiment was significantly higher in comparison to the previous experiment [8], although the concentration of BaP in this experiment was six times lower than the BaP concentration chosen previously. This fact could support a theory about the competitive adsorption processes on PET surface among individual PAH compounds, including BaP.

In commercial rapeseed oil, however, there are also other compounds (*e.g.* vitamins, sterols, *etc.*), which could also interfere with BaP during diffusion or adsorption on the PET surface, and effect the extent of BaP removal from liquid media. To confirm this hypothesis, the rapeseed oil

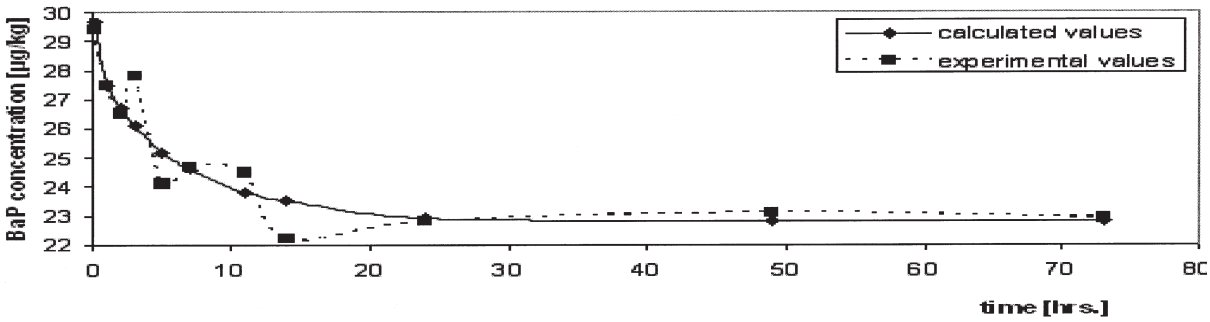

Fig. 1. Changes in BaP concentration in rapeseed oil stored in PET receptacles.

Tab. 1. Parameters calculated from experimentally measured values.

Oil matrix	Diffusion coefficient D [cm^2 h^{-1}]	Equilibrium coefficient β	Viscosity [mPa s]	Temperature during experiment [°C]
Rapeseed oil[†]	4.4×10^{-2}	0.213	[§]	20.4 ± 0.6
Rapeseed oil	2.5×10^{-2}	0.302	65.1	18.3 ± 0.4
Physically refined rapeseed oil	1.8×10^{-1}	0.371	64.7	20.4 ± 0.3
Paraffin oil	6.1	1.201	131	19.7 ± 0.4

[†] from reference [8]
[§] not measured

was physically refined by molecular distillation to separate these compounds from the oil. Although the procedure of physical refining was carried out as carefully as possible, the separation was not complete, as proved by the analysis of vitamin E, the concentration of which was lowered from 203 mg kg^{-1} in the commercial oil to 16.8 mg kg^{-1} in the refined oil, while vitamin A was not detected in both oils. As follows from Fig. 2, a very similar course of BaP concentration decrease was measured on first sight. However, both calculated parameters D and β changed significantly, as shown in Tab. 1. These data suggest that processes taking place in the systems studied indeed have a character of competitive surface adsorption.

To eliminate either effects of residual compounds or eventually also interactions of π-electrons of BaP with lone electron pairs of oxygen, the third sorption experiment was carried out with paraffin oil as a totally non-polar liquid phase. As follows from Fig. 3, the same course of BaP concentration was measured and consequently treated using eq. 1. However, as follows from Tab. 1, both D and β reached significantly highest values, which clearly proves effects of compounds contained in rapeseed oils on the rate of diffusion as well as on the extent of BaP adsorption on the PET surface. As seen in Figs. 1–3, the experimental dependences of BaP concentration *vs.* time exhibit oscillations in BaP concentrations in liquid media, especially at the initial stages of the experiments. A possible effect of temperature fluctuation on the occurrence of oscillations was excluded by temperature monitoring during the experiments. Moreover, the same experimental courses were obtained by two different analytical methods independent of each other – sample preparation and HPLC for rapeseed oils and direct measurement of fluorescence in paraffin oil. Also, the viscosity of all oils studied was very similar (especially when comparing the commercial and refined rapeseed oils). Similar oscillations in concentrations have already been observed in our previous experiments [8], which were originally assigned to "each other" effects of individual PAH compounds.

Taking into account the latest results, it seems that there is a high probability of the existence of an unknown mechanism in the studied systems, which is able to bring about oscillations in BaP concentrations around equilibrium curves calculated on the basis of a generally accepted known theory of adsorption, especially at the initial stages of the experiments.

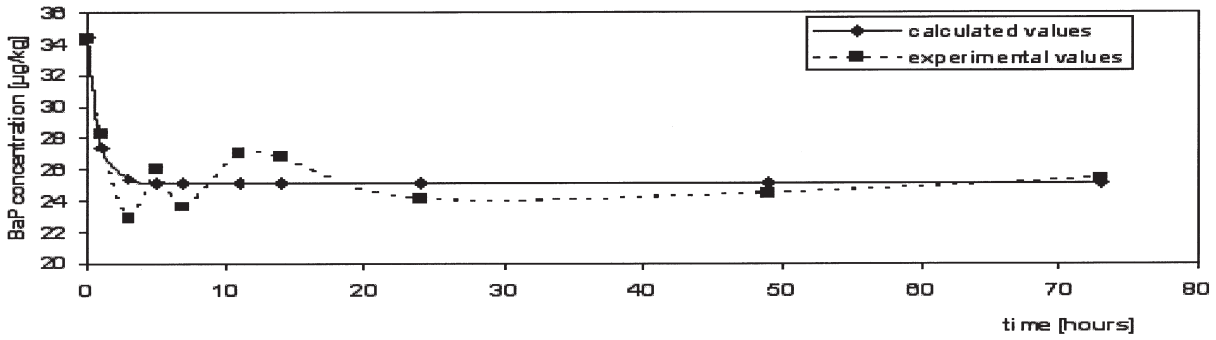

Fig. 2. Changes in BaP concentration in physically refined rapeseed oil stored in PET receptacles.

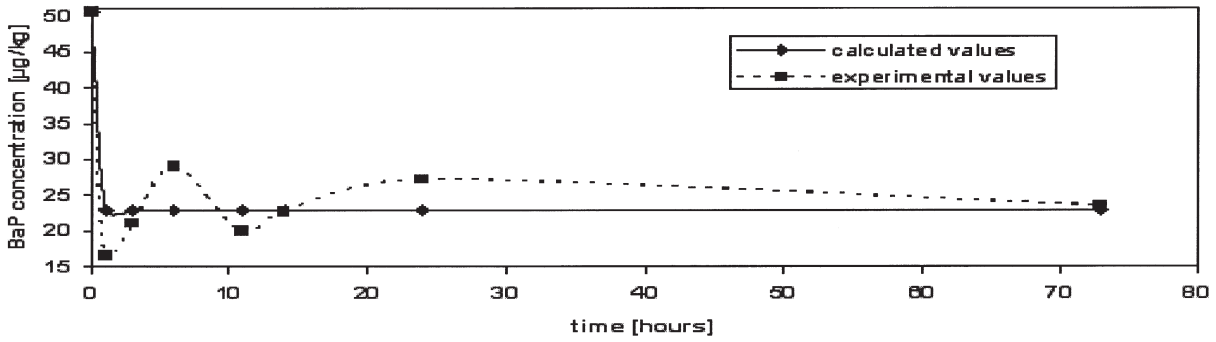

Fig. 3. Changes in BaP concentration in paraffin oil stored in PET receptacles.

Conclusions

The results and findings of this work lead to the following conclusions:

1. The BaP concentration can be decreased by the interaction between BaP contained in rapeseed oil as a liquid phase and PET as a solid phase.

2. The rate of BaP diffusion in the liquid phase and the extent of its adsorption onto PET depend on the presence of other compounds contained in the oil.

3. These compounds are able to either compete with BaP for adsorption centres on the PET surface or interact with BaP itself, which brings about a retardation of the rate of diffusion in liquid media or a lower extent of BaP sorption itself.

4. Considering the courses of BaP concentrations, the mechanism of their oscillations cannot be sufficiently described by the current theory of adsorption.

5. For this reason, the next step of research will be devoted to the study of BaP behaviour in fully defined non-polar liquid media under various physical conditions, to clarify exactly the mechanism of oscillation in BaP concentrations.

6. The interactions between the PET package and foods, in general, are suitable from the view of consumer health protection to decrease the real exposure of human organisms to carcinogenic compounds, which is also important from the point of a correct approach to risk analysis associated with food consumption.

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