

The GC-MS analysis of polycyclic aromatic hydrocarbons content in selected fruit and herbal teas

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Summary

The qualitative and quantitative analysis of polycyclic aromatic hydrocarbons contamination in selected popular brands of fruit and herbal teas was performed using GC-MS method. Among all teas similar quality profiles of PAHs were observed. In all determined compounds 4 light PAHs (phenanthrene, anthracene, fluoranthene, pyrene) from the group of PAHs listed by EPA were predominant. From group of 15 PAHs, marked out for analysis by The UE Scientific Committee on Food, only benzo[a]anthracene, chrysene, 5-methylchrysene, benzo[b]fluoranthene and benzo[k]fluoranthene were stated, although, their levels were relatively low. Benzo[a]pyrene and the most carcinogenic PAHs, namely dibenzopyrenes, were not detected. Moreover, the GC-MS analysis revealed significant differences in contamination level of particular brands of teas among all fruit and herbal teas under investigation.

Key words: polycyclic aromatic hydrocarbons (PAHs), fruit teas, herbal teas, GC-MS

INTRODUCTION

Properties of leaf teas as well as fruit and herbal teas and their health effects are the topics of many scientific researches. It has been proved that tea consumption might have health promoting properties, especially anti-oxidative effects [1-5]. However, agricultural products, and therefore the raw material of fruit and herbal teas could be contaminated with certain undesirable compounds which might

be really dangerous for human health. Polycyclic aromatic hydrocarbons (PAHs) are the example of such ubiquitous environmental pollutants. Gaseous and particle-bound PAHs present in the air could be deposited and accumulated during vegetation [6, 7]. Moreover, essential oils, very common lipophilic compounds of plants which are also present in fruit and herbal teas could contribute to the accumulation of hydrophobic PAHs [8]. It is known that the contamination of teas with PAHs could be not only a result of the raw materials' environmental pollution but also a consequence of drying process [7]. The above-mentioned factors could also diversify the level of PAHs content both in fruit and herbal teas. The present state of knowledge about PAHs, indicating their genotoxic, mutagenic and carcinogenic properties [9], cause that according to the European Commission Recommendation 2005/108/EC from 4 February 2005 [10], further analyses of benzo[a]pyrene and other genotoxic compounds from the 15 PAHs, listed by The Scientific Committee on Food (SCF), are necessary in foodstuffs.

The aim of this research was to study the level of contamination of selected popular brands of fruit and herbal teas with PAHs (including 4 compounds from the group of light PAHs marked out for the analysis by EPA and 15 PAHs listed by The Scientific Committee on Food) by GC-MS method.

MATERIALS AND METHODS

Different popular brands of fruit and herbal teas of Polish producers were purchased at supermarkets in Warsaw agglomeration. From the group of fruit teas the investigated material were teas from rosehip (*Rosa canina*), cranberry (*Oxycoccus palustris*), bilberry (*Vaccinium myrtillus*) and raspberry (*Rubus idaeus*). Among the herbal teas: lime (*Tilia cordata*), pansy (*Viola wittrockiana*), mint (*Mentha longifolia*) and lemon balm (*Melissa officinalis*) teas were taken for the research. Three samples of each product were taken for the study. Moreover, all three samples of the same product were analysed in three repetitions.

The methodology used for the study consisted of PAHs extractions from fruit and herbal teas, extract's cleaning up from interfering compounds by gel permeation chromatography (GPC) and qualitative-quantitative PAHs determination by gas chromatography coupled with mass spectrometry (GC-MS).

The sample of fruit or herbal tea (10 g) homogenized with anhydrous sodium sulphate (20 g) was transferred into an Erlenmeyer flask with 100 cm³ of hexane:acetone (60:40, v/v). Then, the flask was placed into the ultrasonic bath for 30 min. Subsequently, the extract was filtered through a layer of anhydrous sodium sulphate. The obtained filtrate after solvent evaporation was dissolved in 5 cm³ cyclohexane:ethyl acetate (50:50, v/v) and filtered through the polytetrafluoroethylene (PTFE) filter with a pore size of 1 μm. For the step of PAHs isolation clean-up procedure carried out by gel permeation chromatography (GPC) and column TSK Gel G1000HXL (300 x 7.8 mm, 5 μm) were applied. For that purpose 1 cm³ of

the extract obtained previously was injected onto the column. Chromatographic separation was performed by isocratic method with mixture cyclohexane:ethyl acetate (50:50, v/v) used as mobile phase. Samples were eluted at a flow rate of mobile phase of $0.8 \text{ cm}^3 \cdot \text{min}^{-1}$. Afterwards, the purified extract of PAHs was subjected to concentration, then dissolved in toluene and analysed by GC-MS method. The Shimadzu GCMS-QP 2010 equipped with a gas chromatograph GC-2010 and mass spectrometer GCMS-QP 2010 was employed for the analysis. Operating conditions were as follows: 30 m x 0.25 mm i.d. capillary column with film thickness of $0.25 \mu\text{m}$ (ZB-5ms Zebron, Phenomenex); the flow of helium carrier gas was $0.74 \text{ cm}^3 \cdot \text{min}^{-1}$; injector temperature 265°C , splitless injection mode, injection volume $1 \mu\text{l}$; the ion source and interface temperature: 230 and 270°C , respectively. The GC oven temperature was programmed from initial 92°C up to 315°C . The quadrupole analyser measured the abundance of ions of m/z from 100 to 400 and detector voltage was 1.5 kV. Electron ionization (70 eV) with selected ion monitoring mode was used, and the two most abundant ions from the molecular ion cluster were measured for each analysed compound. PAHs identification was performed both on the basis of GC retention time comparison with available PAHs standard solutions and using characteristic ions monitored in analyses.

The standard mixture of 15 PAHs from the list of SCF (PAH-Mix 183, Dr. Ehrenstorfer): cyclopenta[c,d]pyrene (C[cd]p), benzo[a]anthracene (B[a]a), chrysene (Chr), 5-methylchrysene (5-MChr), benzo[j]fluoranthene (B[j]f), benzo[b]fluoranthene (B[b]f) and benzo[k]fluoranthene (B[k]f), benzo[a]pyrene (B[a]p), dibenzo[a,h]anthracene (D[ah]a), dibenzo[a,l]pyrene (D[al]p), benzo[g,h,i]perylene (B[ghi]p), indeno[1,2,3-cd]pyrene (I[cd]p), dibenzo[a,e]pyrene (D[ae]p), dibenzo[a,i]pyrene (D[ai]p) and dibenzo[a,h]pyrene (D[ah]p) and 16 PAHs by EPA (PAH-Mix 9, Dr. Ehrenstorfer) were purchased at Witko (Łódź, Poland). The mixture of 16 PAHs by EPA served at the determination of 4 light PAHs were: phenanthrene (Phen), anthracene (Anthr), fluoranthene (Flu) and pyrene (Pyr).

In accordance with the Commission Regulation (EC) No. 333/2007 performance criteria for methods of analysis for benzo[a]pyrene in foodstuffs are as following: LOD less than $0.3 \mu\text{g}/\text{kg}$, LOQ less than $0.9 \mu\text{g}/\text{kg}$, precision – HORRAT_r values of less than 2 and recovery from 50 to 120% [11]. The measured validation parameters unambiguously proved that the applied method for the determination of 19 PAHs, together with 15 PAHs from the list of SCF, except from those fulfilling all European Union requirements laying down the methods of benzo[a]pyrene analysis in foodstuffs. In addition, they demonstrated satisfactory validation parameters for all remaining PAHs.

Mean contents of the determined 19 PAHs in analysed fruit and herbal teas were expressed in $\mu\text{g} \cdot \text{kg}^{-1}$ of the product. The obtained results were statistically tested using Statistica 7.1 programme. To appraise the significance of differences between means of 19 PAHs content in the group of fruit and herbal teas, the method of multiple comparisons using Tuckey's test was applied, at a level of significance $\alpha=0.05$.

RESULTS AND DISCUSSION

The data concerning mean content of individual PAHs as well as summarized content of 19 PAHs in selected fruit and herbal teas analysed with the use of the GC-MS method are presented in table 1.

Table 1.

The content of polycyclic aromatic hydrocarbons (PAHs) in selected fruit and herbal teas ($\mu\text{g} \cdot \text{kg}^{-1}$, mean \pm SD)

PAH	rosehip tea	cranberry tea	bilberry tea	raspberry tea	lime tea	pansy tea	mint tea	lemon balm tea
Phen	6.43 \pm 0.89	29.15 \pm 4.00	9.82 \pm 0.86	2.74 \pm 0.29	28.99 \pm 3.75	39.15 \pm 4.69	25.69 \pm 2.44	34.31 \pm 2.65
Anthr	0.53 \pm 0.08	2.29 \pm 0.29	4.05 \pm 0.29	0.19 \pm 0.04	4.97 \pm 0.22	5.64 \pm 0.23	1.50 \pm 0.39	3.41 \pm 0.38
Flu	6.00 \pm 0.60	26.02 \pm 2.80	5.57 \pm 0.25	2.00 \pm 0.37	41.08 \pm 4.22	34.16 \pm 4.71	12.54 \pm 1.50	25.16 \pm 3.02
Pyr	2.37 \pm 0.23	6.01 \pm 1.13	2.42 \pm 0.31	1.61 \pm 0.36	20.38 \pm 2.06	5.44 \pm 0.95	7.80 \pm 0.62	16.92 \pm 1.24
C[cd]p	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B[a]a	0.55 \pm 0.11	4.95 \pm 0.45	1.23 \pm 0.41	0.48 \pm 0.17	4.48 \pm 0.43	4.73 \pm 0.49	2.75 \pm 0.36	3.57 \pm 0.51
Chr	1.27 \pm 0.29	6.70 \pm 1.34	1.07 \pm 0.18	0.57 \pm 0.17	8.58 \pm 1.63	7.99 \pm 0.41	4.47 \pm 0.47	3.88 \pm 0.42
5-MChr	n.d.	n.d.	n.d.	1.05 \pm 0.10	n.d.	n.d.	2.08 \pm 0.34	n.d.
B[j]f	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B[b]f	0.34 \pm 0.18	0.69 \pm 0.15	0.36 \pm 0.15	n.d.	1.75 \pm 0.46	1.19 \pm 0.28	0.78 \pm 0.29	0.40 \pm 0.14
B[k]f	0.25 \pm 0.18	1.22 \pm 0.21	0.36 \pm 0.19	n.d.	2.20 \pm 0.59	1.83 \pm 0.40	0.93 \pm 0.32	0.92 \pm 0.32
B[a]p	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
D[ah]a	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
D[al]p	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B[ghi]p	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
I[cd]p	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
D[ae]p	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
D[ai]p	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
D[ah]p	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Σ PAHs	17.75 \pm 1.30 ^{b1}	77.03 \pm 10.37 ^{d1}	24.89 \pm 1.46 ^{c1}	8.64 \pm 0.78 ^{a1}	112.43 \pm 6.70 ^{c2}	100.12 \pm 9.55 ^{bc2}	58.52 \pm 6.74 ^{a2}	88.57 \pm 5.14 ^{b2}

n.d. – not detected

The mean values marked with different letters by the same number (within one from 2 comparisons of total content of PAHs) indicate statistically significant difference between means at a level of $\alpha=0.05$.

The occurrence of phenanthrene, anthracene, fluoranthene, pyrene (light PAHs listed by EPA) and benzo[a]anthracene, chrysene, 5-methylchrysene, benzo[b]fluoranthene and benzo[k]fluoranthene (from the group of 15 PAHs marked out by The Scientific Committee on Food) in analysed teas were stated in the quality profiles of PAHs content. Other heavy PAHs, such as cyclopenta[c,d]pyrene, benzo[j]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene, benzo[g,h,i]perylene, indeno[1,2,3-cd]pyrene

and compounds from the group of dibenzopyrenes were not detected in any of analysed samples of both fruit and herbal teas. It was stated that light PAHs (phenanthrene, anthracene, fluoranthene, pyrene) constituted from 76 to 90% of all determined polyarenes. The percentage of heavy PAHs, for which genotoxic, mutagenic and carcinogenic properties have been stated, was very low in total content of PAHs. Therefore, the obtained qualitative and quantitative PAH profiles confirmed unambiguously that the contamination of analysed teas with PAHs are a consequence of the environment pollution. Similar results have been also stated in other scientific researches [7, 12, 13].

On the basis of performed statistical analysis, significant differences in the total 19 PAHs concentrations were found between individual teas, both in the group of fruit and herbal teas.

Statistically, among the fruit teas the lowest total PAH content, $8.64 \mu\text{g} \cdot \text{kg}^{-1}$, was determined in raspberry tea (*R. idaeus*), whereas the highest level was observed for cranberry tea (*O. palustris*), in which mean content of 19 PAHs was $77.03 \mu\text{g} \cdot \text{kg}^{-1}$. The raw material of this tea is a kind of red berry with the peel covered with the wax layer. It is obvious that lipophylic compounds contribute to accumulation of hydrophobic PAHs in the product and, in consequence, influence the higher PAH contamination level in comparison to other fruit teas. In bilberry (*V. myrtillus*) and rosehip tea (*R. canina*), total concentration of 19 PAHs was about $24.89 \mu\text{g} \cdot \text{kg}^{-1}$ and $17.75 \mu\text{g} \cdot \text{kg}^{-1}$, respectively.

In herbal teas the lowest level of 19 PAHs contamination was determined in mint tea (*M. longifolia*), at the level of $58.52 \mu\text{g} \cdot \text{kg}^{-1}$. However, the highest sum of 19 PAHs amounting $112.43 \mu\text{g} \cdot \text{kg}^{-1}$ was stated for lime tea (*T. cordata*). It was also statistically proved that pansy tea (*V. wittrockiana*) had insignificantly lower total level of PAHs contamination than lime tea. Moreover, the mean content of 19 PAHs in the herbal teas ($89.91 \mu\text{g} \cdot \text{kg}^{-1}$) was more than two times higher than in the fruit teas. It is known that leaves morphology and their physiological properties as well as the proximity of environmental sources have considerable influence on the level of PAHs contamination [14]. As an example, leaves of mint, which are longitudinally oval-shaped, have particularly large amounts of essential oils (1.5–3.5%). Moreover, they are hairy and have well-developed respiratory system. However, the lemon balm leaf has considerably lower level of essential oils content (about 0.1%, mainly citral), but being more hairy and having larger surface could favour polyarenes pollution. Considering the high level of PAHs contamination in pansy tea, it could be also the result of the raw material specificity. The pansy leaves are hairy, too. Moreover, they are filled with mucilage [15] which could influence the level of accumulation of hydrophobic PAHs. Considering the lime tea contamination, it should be added that small-leaved lime (*T. cordata*) is a very popular ornamental tree grown throughout its native range, along streets and roads. Unlike mint and lemon balm, lime inflorescence used for lime tea production is therefore taken from natural habitats, so the level of PAHs content in lime tea is directly and strongly dependent on the raw material environmental contamination.

In the view of the obtained results, it ought to be pointed out that polyarenes content in analysed fruit and herbal teas vary in considerable range. It was also confirmed by Lin et al. [7] who stated that in different kinds of teas, including herbal teas, total content of 16 PAHs (from EPA list) ranged from 323 to 8800 $\mu\text{g} \cdot \text{kg}^{-1}$. Wide range of PAHs levels in different brands of herbal and fruit teas was also proved in research of Schlemitz and Pfannhauser [8]. The lowest level of 16 EPA PAHs contamination was found in dill tea and multifruit tea (13.41 and 17.53 $\mu\text{g} \cdot \text{kg}^{-1}$, respectively). Studies dealing with PAHs content in selected fruit and herbal teas conducted by Ciemniak [13] indicated that the lowest 16 PAHs content (from EPA list) was found in hibiscus tea (48.23 $\mu\text{g} \cdot \text{kg}^{-1}$). Results similar to these obtained in our research, the most contaminated was lime tea, in which the level of 16 PAHs content amounted 1040.47 $\mu\text{g} \cdot \text{kg}^{-1}$. Moreover, the high level of light PAHs in the total polyarenes content was similar to ours [13].

CONCLUSIONS

The obtained results indicated that PAHs content in particular brands of fruit as well as herbal teas vary in considerable range. Moreover, the applied method revealed similar quality profiles of PAHs with predominant light PAHs. That suggests that contamination of analysed teas is the consequence of the environmental contamination of their raw materials.

This research was performed with the financial support of Polish State Committee for Scientific Research (KBN) under grant No. 501 0928 00 29.

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ANALIZA GC-MS ZAWARTOŚCI WIELOPIERŚCIENIOWYCH WĘGLOWODORÓW AROMATYCZNYCH W WYBRANYCH HERBATEKACH OWOCOWYCH I ZIOŁOWYCH

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Streszczenie

Analizę jakościowego i ilościowego zanieczyszczenia wielopierścieniowymi węglowodarami aromatycznymi wybranych popularnych gatunków handlowych herbatek owocowych i ziołowych przeprowadzono przy zastosowaniu metody GC-MS. Wśród wszystkich herbatek zaobserwowano zbliżone profile jakościowe zawartości WWA. W sumarycznej zawartości oznaczanych związków dominujący udział stanowiły 4 lekkie WWA (fenantren, antracenu, fluorantenu, piren) z grupy WWA wytypowanych do badań przez EPA. Z grupy 15 WWA, wytypowanych do badań przez Komitet Naukowy ds. Żywności UE, stwierdzono występowanie benzo[a]antracenu, chryzenu, 5-metylochryzenu, benzo[b]fluorantenu i benzo[k]fluorantenu, jednakże poziomy ich zawartości były względnie niskie. Nie wykryto obecności benzo[a]pirenu oraz najbardziej kancerogennych WWA, a więc dibenzopirenów. Ponadto analiza GC-MS wykazała istotne zróżnicowanie poziomów zanieczyszczenia poszczególnych gatunków analizowanych herbatek owocowych i ziołowych.

Słowa kluczowe: wielopierścieniowe węglowodory aromatyczne (WWA), herbatki owocowe, herbatki ziołowe, GC-MS