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Polycyclic Aromatic Hydrocarbons in Popcorn Corn Varieties and Popcorns

Kristina Habschied [1,*], Vinko Krstanović [1], Brankica Kartalović [2], Vlatko Galić [3], Tatjana Ledenčan [3], Krešimir Mastanjević [1,*] and Zvonimir Zdunić [3]

1 Faculty of Food Technology, Josip Juraj Strossmayer University of Osijek, 31000 Osijek, Croatia
2 Scientific Veterinary Institute Novi Sad, Rumenački put 20, 21000 Novi Sad, Serbia
3 Agricultural Institute Osijek, Južno predgrađe 17, 31000 Osijek, Croatia
* Correspondence: khabschi@ptfos.hr (K.H.); kmastanj@gmail.com (K.M.);
Tel.: +385-31-490-411 (K.H.); +385-31-224-373 (K.M.)

Abstract: Popping corn is a widespread activity and popcorns are one of the most consumed snacks in the world. As popcorn corn is exposed to environmental conditions and agrotechnical measures in the field, polycyclic aromatic hydrocarbons (PAHs) can end up in corn kernels. Since popcorns are popped at higher temperatures, ca. 180 °C, it is reasonable to assume that PAHs can be found in popcorns. The objective of this paper was to preliminarily determine and quantify the incidence of various PAHs in different popcorn varieties and popcorns popped from them, during two consecutive years. PAHs were determined by using GC-MS. Popcorn corn contained only light PAHs, naphthalene (Nap), acenaphthene (Ane), and fluorene (Fln). However, popped popcorns contained heavier PAHs, such as benz[a]anthracene (BaA), chrysene (Chry), and benzo[a]pyrene (BaP) whose ∑PAH4 was 8.39 μg/kg in sample 1 in 2020. The results indicate that popcorns can be a significant source of PAHs and further studies should be conducted. This is concerning since popcorns are favored snacks in all age groups, including children.

Keywords: PAHs; popcorn corn hybrids; popcorns; GC-MS

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1. Introduction

Zea mays ssp. *Everta* is a widespread corn cultivar, one of the oldest commodities known to humans. The kernel consists of the endosperm, pericarp, and germ, with the endosperm being the most important part in popping corn [1]. The corn cultivar, moisture content in the kernel, storage conditions, type of packaging, etc., all affect the quality of popping popcorn produced in microwave ovens [2]. The grain bursts at temperatures of 150–200 °C, during which the water from the grain changes into steam. It then transforms the endosperm into a white hollow mass of increased volume. At higher pressure, the heated steam converts the soft starch into a gelatinous material. Due to the increase in pressure in the pericarp, the grain bursts. Hoseney et al. [3] state that the first pop has the greatest influence on the popping volume. Approximately 96% of popping occurs at approximately 180 °C, while, at lower temperatures, the popping decreases [4]. At the moment that the grain bursts, the water is overheated. This property leads to the expansion of the grain when the pericarp bursts [5]. The optimal share of moisture in the grain for popping is 10 to 15%. If the moisture content is lower, there is not enough water in the grain, whose steam can create pressure and lead to the complete cracking of the grain. At high moisture content, the pericarp weakens and causes the grain to burst before the appropriate pressure is reached [6]. The maximum bursting potential is realized if the popcorn kernel reaches full maturity, and kernel moisture is the key to optimal bursting. The product created by popping the kernels is called popcorn.

The origin of polycyclic aromatic hydrocarbons (PAHs) is commonly any type of pyrolytic process, such as incomplete wood burning (or other organic matter). Fires, volcanic eruptions, and carbonization cause the PAHs to end up in the environment [7–9]. They can differ by the number of fused rings, and thus can be of lower molecular weight (2–4 rings) (LMW-PAHs) and higher molecular weight (more than 4 rings) (HMW-PAHs). PAHs are lipophilic molecules and acknowledged as health deteriorators [10–12] due to their presence in various foods and beverages. PAHs are designated as persistent organic pollutants (POPs) and are commonly found in different commodities. Crops are usually contaminated via soil, water, or air, since PAHs are ubiquitous and highly mobile, thus representing a serious environmental threat [13–15]. According to Mackiewicz-Walec et al. [16], plants have the ability to secrete enzymes and transform or biodegrade PAHs in soil. In doing so, some plants show more effectiveness in the biodegradation of PAHs than other species.

Foods, especially processed samples, are complex materials for analysis. Usually, analytes have to be separated from the matrix. Since PAHs are lipophilic molecules, upon extraction, they merge with the lipid constituents and have to be purified. Thus, a number of different approaches have been developed to aid the removal of lipid components from the matrix. They involve solid or liquid extractions or liquid–liquid extraction methods, pressurized liquid extraction, or even supercritical fluid extraction. After purification, the partition methods, such as solid-phase extraction or solid-phase microextraction, follow. At the end, analytical determination is usually performed by applying liquid chromatography (LC), ultra-high-performance LC (UHPLC), or gas chromatography (GC) with different detectors. Recent studies employ comprehensive techniques such as GC × GC or LC × LC. A deeper overview of extraction and analytical methods can be found in excellent review papers [17–19].

Current reports indicate that data on PAHs in cereals are lacking. Not many researchers have dealt with PAHs in cereals. However, some of them have been determined in breakfast cereals and bread [20] and dried maize grain samples [21]. According to Bertinetti et al. [22], the levels of PAHs in rice are in relation to the source of heating. Another research work [23] investigated the influence of the drying process on the levels of PAHs in granola, chocolate granola, and milk-filled cereals. However, there are no data on PAH content in popcorns. Since popcorn corn is subjected to heat via the process of roasting, similar to coffee or cocoa beans, it is reasonable to assume that PAHs can be formed during the preparation of popcorns.

Due to different levels of carcinogenic activity, the European Food Safety Authority (EFSA) has set limits for the levels of benzo[a]pyrene and the sum of the concentrations of four PAHs (benzo[a]pyren, benz[a]anthracene, benzo[b]fluoranthene, and chrysene (PAH4) [8]) as the main indicators of PAH toxicity through foodstuffs. According to the regulation [24], the maximum allowed level of benzo[a]pyrene in processed cereal-based foods should not exceed 1 μg/kg, and the ∑PAH4 levels should not exceed 1 μg/kg.

Since there are no data on PAHs in popcorns, this research aimed to investigate whether popping corn can result in PAHs in popcorns, and to report and quantify the occurrence of different PAHs in different popcorn varieties and the popcorns popped from them during two consecutive years using a microwave oven. The used method for analysis was GC × MS, described in detail in the Supplementary Material.

2. Materials and Methods

2.1. Sample Preparation

Popcorn corn hybrids were sown on the plots of the Osijek Agricultural Institute (OAI) in two repetitions in a completely randomized design in 2020. Sowing was carried out at the end of April 2020 and 2021. Manual harvesting was carried out when grain moisture was lower than 20% (October). The hybrid samples were dried in a dryer at 40 °C for 24 h.

Hybrids:

1. Dh2xOS671;
2. Dh2xOS674;
3. Dh2xOS691;

4. OS12xDh7-3;
5. Standard–Bulut;
6. OS20Xos691.

New experimental OAI hybrids (1, 2, 3, 4, 6) were used, in addition to one PIO commercial standard (5).

Popcorns were prepared from 100 g of dry grain (<14% moisture) in a microwave oven (Electrolux, Stockholm, Sweden) in a closed special bag (Kolysen Packaging Integration Co., Ltd., Xiamen, China) for 120 s at 700 W without using oil.

The expanded, unopened bag was placed in the middle of the microwave oven on the rotary plate, paying attention to the instruction "this side up". The oven was set to the strongest degree of heating (700 W), in a time range of 2.5 min, so that the popcorn did not burn. Before opening, the bag was thoroughly shaken (so that the contents were evenly distributed) and opened by pulling the opposite ends of the bag, paying attention to the escaping steam. Uncracked corn and the bag could no longer be used. The microwave oven was cleaned with acetone after every use, to minimize the possibility for PAH carryover from one sample to another.

Popcorn corn and popcorns were milled on a laboratory knife mill (Grindomix GM 200, Retsch GmbH, Haan, Germany). Three grams were weighed and subjected to PAH analysis as described in the Supplementary material.

2.2. PAH Analysis

PAH analysis was carried out as described by Mastanjević et al. [25] using a gas mass chromatography device (Agilent 7890B/5977A MSD, Santa Clara, CA, USA). A DB-5MS column (30 m × 0.25 µm × 0.25 mm) (Agilent J&W, Santa Clara, CA, USA) was used in the analysis. Calibration, sample preparation (QuEChERS) and validation, as well as the GC operating conditions, are described in detail in the Supplementary Material.

2.3. Statistical Analysis

Analysis of variance (ANOVA) and Fisher's least significant difference test (LSD) were carried out and a p value < 0.05 was set to be significant. Statistical analysis was performed using Statistica 13.1. (TIBCO Software Inc., Palo Alto, CA, USA).

3. Results and Discussion

Since popcorn corn is exposed to environmental conditions and agrotechnical measures in the field, it is reasonable to assume that PAHs can be found in popcorn corn. The results of this research analysis showed that a certain type and quantity of PAHs can be detected in popcorn corns. Namely, Table 1 shows the concentrations of PAHs in popcorn corn, and it is evident that the amounts of PAHs mostly comprised light PAHs.

Table 1. PAH content (µg/kg) in different popcorn corn varieties in year 2019.

	Sample					
PAH	**1**	**2**	**3**	**4**	**5**	**6**
Nap	$2.58^{b} \pm 0.04$	$1.78^{c} \pm 0.10$	$3.63^{a} \pm 0.02$	$0.27^{e} \pm 0.02$	-	$1.34^{d} \pm 0.02$
Ane	$0.35^{a} \pm 0.10$	$0.22^{b} \pm 0.06$	$0.23^{b} \pm 0.07$	-	-	-
Fln	$0.55^{b} \pm 0.01$	$0.56^{b} \pm 0.05$	$0.51^{b} \pm 0.08$	$1.02^{a} \pm 0.10$	-	$1.13^{a} \pm 0.08$
∑PAH16	$3.48^{b} \pm 0.08$	$2.56^{c} \pm 0.07$	$4.37^{a} \pm 0.11$	$1.29^{d} \pm 0.07$	-	$2.47^{c} \pm 0.08$

$^{a–e}$ Means ± standard deviation within rows with different superscripts are significantly different (p < 0.05); - below limit of quantification.

Detected light PAHs in this research, for both years (Tables 1 and 2), were Nap, Ane, and Flu. The highest concentration of Nap was detected in sample 3, with 3.63 ± 0.02 µg/kg, in 2019, while the highest concentration of Flu was detected in the same sample in 2020 (7.22 µg/kg). From the results, it appears that the standard hybrid Bulut (sample 5) showed the highest resistance to PAHs in popcorn corn, especially in 2019. This is probably related to the amount of fat in popcorn corns.

Table 2. PAH content (μg/kg) in different popcorn corn varieties in year 2020.

	Sample					
PAH	**1**	**2**	**3**	**4**	**5**	**6**
Nap	0.43 ^{b} ± 0.03	3.22 ^{a} ± 0.05	-	0.16 ^{d} ± 0.01	-	0.34 ^{c} ± 0.01
Ane	0.35 ^{a} ± 0.10	-	-	-	-	-
Fln	3.23 ^{b} ± 0.01	1.26 ^{c} ± 0.05	7.22 ^{a} ± 0.04	0.53 ^{d} ± 0.01	3.24 ^{b} ± 0.01	-
∑PAH16	4.00 ^{c} ± 0.01	4.48 ^{b} ± 0.05	7.22 ^{a} ± 0.04	0.69 ^{e} ± 0.01	3.24 ^{d} ± 0.01	0.34 ^{f} ± 0.01

$^{a–f}$ Means ± standard deviation within rows with different superscripts are significantly different ($p < 0.05$); - below limit of quantification.

The lack of heavy PAHs can probably be attributed to the hard and waxy coat of the maize kernel, which retains some of the lighter environmental PAHs (during cultivation and growing). Although there are not many similar studies conducted on popcorn corn, nor corn in general, these results can be compared with the research conducted by Kiš et al. [26], in which no PAHs were detected in corn grains before drying, or with the work of Muntean et al. [27], who also conducted a similar study. They conducted PAH analysis for three years, during which they detected mostly light PAHs in corn kernels, i.e., Nap, Flu, and Ane. The highest concentration was recorded for Nap, with around 4 μg/kg for each year from a location in Cluj-Napoca. Other PAHs were detected in lower concentrations. The total sum of all detected PAHs in their research was over 7 μg/kg.

On the other hand, the amounts of PAHs in the popcorns analyzed in this research were significant for some PAHs and some samples. Tables 3 and 4 show the amounts of PAHs in popcorn that was popped in a bag.

Table 3. PAH content (μg/kg) in different popcorn varieties in year 2019.

	Sample					
PAH	**1**	**2**	**3**	**4**	**5**	**6**
Nap	4.58 ^{c} ± 0.10	3.20 ^{d} ± 0.11	5.25 ^{a} ± 0.13	2.37 ^{e} ± 0.17	-	5.17 ^{b} ± 0.21
Ane	0.96 ^{a} ± 0.10	0.49 ^{e} ± 0.07	0.79 ^{b} ± 0.09	0.69 ^{c} ± 0.08	0.28 ^{f} ± 0.08	0.54 ^{d} ± 0.10
Acn	-	-	-	-	-	-
Fln	3.18 ^{c} ± 0.19	2.35 ^{e} ± 0.11	4.84 ^{a} ± 0.12	2.77 ^{d} ± 0.15	0.13 ^{f} ± 0.07	4.63 ^{b} ± 0.17
Ant	21.69 ^{c} ± 0.19	13.72 ^{e} ± 0.29	27.54 ^{a} ± 0.21	19.53 ^{d} ± 0.22	3.11 ^{f} ± 0.17	24.93 ^{b} ± 0.18
Phen	8.09 ^{d} ± 0.41	5.56 ^{f} ± 0.61	9.41 ^{b} ± 0.61	8.98 ^{c} ± 0.55	11.62 ^{a} ± 0.75	6.62 ^{e} ± 0.49
Flt	1.90 ^{b} ± 0.15	1.48 ^{d} ± 0.12	2.27 ^{a} ± 0.14	1.90 ^{b} ± 0.11	1.38 ^{e} ± 0.09	1.79 ^{c} ± 0.10
BaA	0.96 ^{a} ± 0.07	-	-	-	0.32 ^{b} ± 0.06	-
Pyr	2.11 ^{a} ± 0.33	0.96 ^{e} ± 0.07	1.49 ^{b} ± 0.17	1.13 ^{d} ± 0.09	1.24 ^{d} ± 0.10	1.24 ^{c} ± 0.11
Chry	0.31 ^{a} ± 0.06	-	-	-	-	-
BbFA	-	-	-	-	-	-
BkFA	-	-	-	-	-	-
BaP	0.25 ^{a} ± 0.10	-	-	-	-	-
DBahA	-	-	-	-	-	-
BghiP	-	-	-	-	-	-
In	-	-	-	-	1.29 ^{a} ± 0.10	-
∑PAH4	1.52 ^{a} ± 0.10	-	-	-	0.32 ^{b} ± 0.06	-
∑PAH16	44.03 ^{c} ± 0.55	27.76 ^{e} ± 0.34	51.59 ^{a} ± 1.48	37.37 ^{d} ± 0.69	19.37 ^{f} ± 0.38	44.92 ^{b} ± 0.91

$^{a–f}$ Means ± standard deviation within rows with different superscripts are significantly different ($p < 0.05$); - below limit of quantification.

Table 4. PAH content (μg/kg) in different popcorn varieties in year 2020.

	Sample					
PAH	**1**	**2**	**3**	**4**	**5**	**6**
Nap	1.97 ^{b} ± 0.09	11.17 ^{a} ± 0.19	<LOQ	1.97 ^{b} ± 0.07	<LOQ	1.00 ^{c} ± 0.10
Ane	2.07 ^{b} ± 0.11	0.58 ^{e} ± 0.09	1.23 ^{d} ± 0.09	0.39 ^{f} ± 0.05	0.28 ^{f} ± 0.08	3.11 ^{a} ± 0.17
Acn	-	-	-	-	-	-
Fln	9.96 ^{a} ± 0.17	6.37 ^{b} ± 0.14	2.63 ^{c} ± 0.10	2.19 ^{d} ± 0.16	0.13 ^{f} ± 0.07	1.91 ^{f} ± 0.22
Ant	69.88 ^{a} ± 1.11	33.68 ^{b} ± 0.89	26.21 ^{c} ± 0.85	16.94 ^{e} ± 0.42	14.93 ^{f} ± 0.21	23.07 ^{d} ± 0.22
Phen	29.25 ^{a} ± 0.22	6.61 ^{c}± 0.23	6.31 ^{bd} ± 0.33	6.21 ^{e} ± 0.27	4.43 ^{af} ± 0.35	10.20 ^{b} ± 0.19
Flt	9.81 ^{ba} ± 0.27	2.71 ^{b} ± 0.09	2.39 ^{d} ± 0.19	1.33 ^{f} ± 0.10	2.20 ^{e} ± 0.15	2.60 ^{c} ± 0.10
BaA	1.25 ^{a} ± 0.08	0.15 ^{b} ± 0.05	-	-	-	-
Pyr	6.01 ^{a} ± 0.43	1.64 ^{b} ± 0.17	1.52 ^{c} ± 0.14	0.88 ^{f} ± 0.07	1.18 ^{e} ± 0.10	1.39 ^{d} ± 0.09
Chry	0.56 ^{a} ± 0.04	-	-	-	-	-
BbFA	-	-	-	-	-	-
BkFA	-	-	-	-	-	-
BaP	6.58 ^{a} ± 0.77	-	-	-	-	-
DBahA	-	-	-	-	-	-
BghiP	-	-	-	-	-	-
In	-	-	7.59 ^{a} ± 0.91	3.21 ^{d} ± 0.11	5.73 ^{c} ± 0.17	5.96 ^{b} ± 0.43
∑PAH4	8.39 ^{a} ± 0.22	0.15 ^{b} ± 0.05	-	-	-	-
∑PAH16	137.34 ^{a} ± 0.75	62.91 ^{b} ± 0.45	47.88 ^{c} ± 0.88	33.12 ^{d} ± 0.55	28.88 ^{d} ± 0.27	49.24 ^{c} ± 0.87

$^{a–f}$ Means ± standard deviation within rows with different superscripts are significantly different ($p < 0.05$); - below limit of quantification.

As can be observed, PAHs that appeared in some samples were Ane, Flu, Ant, Phen, Flt, BaA, and Pyr. The highest concentration was recorded for Ant in sample 1 in year 2020, and it was almost 70 μg/kg, while in sample 2, for the same year, it was 33.68 μg/kg. The PAH that was detected in the next highest concentration was Phen, with 29.25 μg/kg, in sample 1 for 2020. Although these concentrations are relatively high, they are still light PAHs that have a weaker effect on human health and are not included in the legislation. It appears that more PAHs were determined in samples in year 2020 than in 2019. The highest concentration of PAH in year 2019 was detected in sample 3 for Ant and it amounted to 27.54 μg/kg.

Heavier compounds, such as benzo(b)fluoranthene and light chrysene, were also detected in some of the samples, and again in higher concentrations in year 2020. BbFA, BkFA, DBahA, and BghiP were not detected in any of the samples. Chrysene was recorded in sample 1 in 2019 and in a low concentration (0.31 μg/kg), but it contributed to the total concentration of PAH4, so the amount of ∑PAH4 for sample 1 was 1.52 μg/kg. In 2020, samples generally contained higher concentrations of PAHs. Sample 1 in 2020 also contained Chry, at 0.56 μg/kg. However, in this sample, BaA and BaP were also significantly higher than in 2019, which led to a ∑PAH4 amount of 8.39 μg/kg. This value is eightfold higher than the prescribed limit for cereals (EU Regulation No. 835/2011), which is 1 μg/kg [28]. This information could be alarming for consumers and should be paid attention to in terms of legal regulation.

Due to the lack of comparable literature, the results of this research have been correlated with most similar papers regarding corn or other commodities exposed to heating or roasting, such as coffee beans or cocoa beans. Hutt et al. [29] measured PAHs in corn and wheat before and after drying. The ∑PAH4 value for dried corn samples was significantly higher than in wet corn. Chrysene's value was above 32 μg kg^{-1}. The temperature needed for popping popcorns is above 180 °C, which is obviously high enough to form light and heavier PAHs. The addition of oil or butter might serve to increase the content of PAHs in popcorns. This experiment was carried out on popcorn popped in bags without added oil, and, given that popcorn can be prepared in containers with added oil or butter, or in devices for preparing popcorn, it should certainly be investigated whether the method of preparation affects the occurrence of PAHs in popcorns. Bulut, a standard hybrid, showed the lowest levels of PAH16 for both years. The highest levels of PAHs were determined in new hybrid Dh2xOS671, for both years.

The detection of PAHs in popcorn corn can be attributed to environmental contamination (soil, water, and atmosphere) [30], since PAHs are classified as persistent organic pollutants (POPs) [31]. Namely, crops are known to uptake PAHs from contaminated soil. Further consumption of contaminated crops leads to PAH transfer via the food chain [32–35]. The ability of maize plants to degrade PAHs is described in several papers [36–38]. As reported by Lin [36], maize roots and the tops of plants can accumulate PAHs from aqueous solutions and air in proportion to exposure levels. In other research, PAH concentrations determined in maize were below the permissible limit established by the EFSA, and the maize plant has potential for the degradation of phenanthrene and pyrene [37,38]. Some research even describes the use of the maize plant as an alternative technology for the bioremediation of PAH-contaminated soils [39].

However, since popcorn grains contained low amounts of PAHs, it is clear that most of the PAHs in the studied popcorns formed during popping. This is probably due to the exposure to heat during popping, as popcorns are exposed to heating and subsequently the cooking of starch in the kernel until it expands. Although the expansion of the corn kernel is a relatively quick process, it requires a certain amount of energy to be absorbed by the starch in order to expand. PAH formation takes place while food is being exposed to direct heating at high temperatures, resulting in food combustion [40]. This is when free radicals g during food combustion at high temperatures recombine and form light PAHs, followed by heavy PAHs [41]. Ant was present in all samples, which indicates that it is omnipresent. It is a light PAH and can end up in samples via air, water, and soil.

Considering that popcorns are very light, it is possible that these PAH concentration values cannot significantly affect health, but it should be noted because popcorn is a widespread snack consumed by all age groups and very often. For example, the average consumption of popcorns in the USA is 60 quarts per year, which is approximately 56 kg per person per year [42].

4. Conclusions

The results indicate that certain amounts of PAHs can be found in popcorns. However, only light PAHs were detected in the raw material for popcorns, popcorn corn.

Since popcorns are a widespread snack, available to all ages, the results of this research draw attention. Namely, although only small amounts of light PAHs were detected in popcorn corn (Nap, Ane, Flu), popped popcorns contained higher levels of PAHs and more types of PAHs (Nap, Ane, Fln, Ant, Phen, Flt, BaA, Pyr, Chry, BaP). Sample 1 of popped popcorns contained over 8 μg/kg of PAH4 (2020), while the same sample of popcorn corn contained no PAH4. It is evident that the heating of popcorn corn during the preparation of popcorns can result in significant amounts of PAHs in popcorns. In conclusion, popcorns can contain significant concentrations of PAHs, which is concerning since they are available to all age groups. Further studies should include the application of different popping corn methods with the addition of oil and salt.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app13053107/s1, Table S1: The average values for precision, reproducibility, accuracy, linearity, LOQ and LOD for PAH method validation.

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