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Communication

Polycyclic Aromatic Hydrocarbons in Malt

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Abstract: The kilning of malt occurs at different temperatures, depending on the desired color and aromas. Higher temperatures applied during kilning can be involved in polycyclic aromatic hydrocarbons (PAHs) formation in malt. PAHs are undesirable and designated as health hazards, it is important to quantify and qualify them in different malts. Since the European Food Safety Authority (EFSA) gave strict recommendations about PAHs in different foods, but omitted malt as a potential hazardous raw material that can cause health damage to beer consumers, the aim of this investigation was to assess the presence of 16 PAHs (naphthalene (Nap), acenaphthylene (Anl), acenaphthene (Ane), fluorene (Flu), anthracene (Ant), phenanthrene (Phen), fluoranthene (Flt), benz[*a*]anthracene (BaA), pyrene (Pyr), chrysene (Chry), benzo[*b*]fluoranthene (BbF), benzo[*k*]fluoranthene (BkF), benzo[*a*]pyrene (BaP), dibenz[*a,h*]anthracene (DahA), benzo[*ghi*]perylene (BghiP), and indeno[1,2,3-*cd*]pyrene (InP)) in different, commercially available malts (amber, black, pilsner, and cara-120). The results showed that PAHs are present in different malts, with some in high amounts (BaA in black malt was 737 μg/kg). Minimal levels of BaA were detected in the amber malt, 60.53 μg/kg. The PAH4 (BaP, BaA, BbF, and Chry) sums are identical to the BaA concentrations in all malts and greatly exceed the EFSA prescribed levels for PAH4 in processed cereal-based foods (1 μg/kg).

Keywords: malted barley; kiln; PAH16; PAH4; GC-MS

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1. Introduction

Beer is a widely consumed alcoholic drink in all parts of the world. The basic raw material for beer production is malt. Different beer styles demand different malt types, which are a result of applied kilning temperatures. Besides beer, malt flour can be added to various breads and baking products as a color enhancer and for its enzymatic activity. This could lead to an increased concentration of polycyclic aromatic hydrocarbons (PAHs) in bread and baking products, which are consumed in all areas of the world, as well [1]. Malted cereals are a basic material in whiskey production, too. All these products can contain different amounts of PAHs that originate from malt.

PAHs are found in many food samples, and their occurrence is reported in many papers. They originate from incomplete wood combustion during smoking and present a serious health hazard to humans [2,3]. Polycyclic aromatic hydrocarbons (PAHs) are also present in the environment, as a consequence of the incomplete combustion of fossile fuels [4]. They can be found in tobacco smoke, in high concentrations. Char-broiling and smoking of foods greatly affects PAH levels in food and beverages [4].

PAH molecules consist of two or more fused aromatic rings [5]. PAHs show different degrees of carcinogenicity but, in general, they all contribute to the carcinogenicity of other PAHs [6]. PAH molecules display lipophilic properties and can be found in different foods that contains fats [7–9]. In general, PAH concentration in foods varies, due to different factors, such as the technique of smoking, choice of wood, duration of smoke exposure, and type of food itself [10–12]. However, to reduce the PAH concentrations in foods, Codex Alimentarius [13] recommends optimal smoking exposure. There are other recommended methods for PAH concentration reduction in foods. They include marinading (beer, spices, garlic, and onion can inhibit the formation of PAHs in meat) [14–17], irradiation [18], the washing of smoked fish [17], and the reduction of smoke contamination [3,7,9], cooking methods (application of lower temperatures, exposing the lean part of the meat while grilling, and avoiding flame using electrical grills instead of charcoal) [2,19,20]. In research conducted so far, PAHs have been identified in breakfast cereals and bread [21]. The authors reported that BaP and PAH4 concentrations were below the prescribed limit specified by the European legislation (1 μg/kg) [21,22]. Rascón et al. [23] correlated the process of drying with levels of PAHs in products such as granola, chocolate granola, and milk-filled cereal. According to Rascón et al. [24], beer samples contained naphthalene (340–1500 ng/L) and anthracene (320–2200 ng/L).

As mentioned before, different PAHs express different carcinogenic properties, but they all contribute to the general carcinogenic image of certain smoked foods. Because of that, the European food safety authority (EFSA) determined that the concentrations of benzo[*a*]pyrene (BaP) and the sum of the concentrations of four PAHs (benzo[*a*]pyrene (BaP), benz[*a*]anthracene (BaA), benzo[*b*]fluoranthene (BbF), and chrysene (Chry) (PAH4) [25]) would be considered the main indicators of PAHs toxicity via foods. According to the European commission (EU) regulation no. 835/2011 [22], the maximum permissible concentration of BaP in processed cereal-based foods products is 1 μg/kg, and the sum of PAH4 concentrations is also 1 μg/kg. There are many studies considering the carcinogenic potential and occurrence of PAHs in food products, especially in smoked meat products [3,8,26,27], but there is a lack of information about PAH occurrence in malt and malt-related products (foods and beverages). Only a few papers describe PAH presence in beer [16,24,28] and malt [29–31]. According to IARC (International Agency for Research on Cancer) [32], based on their carcinogenic properties, PAHs can be divided into several groups, shown in Table 1. Figure 1 shows the chemical structures of analyzed PAHs.

Table 1. Evaluation of the polycyclic aromatic hydrocarbons, according to IARC [32].

Polycyclic Aromatic Hydrocarbon (PAH)	Abbreviation	IARC Carcinogenic Group *
benzo[*a*]pyrene	BaP	1
dibenz[*a,h*]anthracene	DahA	2A
Naphthalene	Nap	2B
benz[*a*]anthracene	BaA	
Chrysene	Chry	
benzo[*b*]fluoranthene	BbF	
benzo[*k*]fluoranthene	BkF	
indeno[1,2,3-*cd*]pyrene	InP	
acenaphthene	Ane	3
fluorene	Fln	
phenanthrene	Phen	
anthracene	Ant	
fluoranthene	Flt	
pyren	Pyr	
benzo[*g,h,i*]perylene	BghiP	
acenaphthylene	Ane	4

* Group 1—carcinogenic to humans; group 2A—probably carcinogenic to humans; group 2B—possibly carcinogenic to humans; group 3—not classifiable, in regards to its carcinogenicity to humans; group 4—probably not carcinogenic to humans.

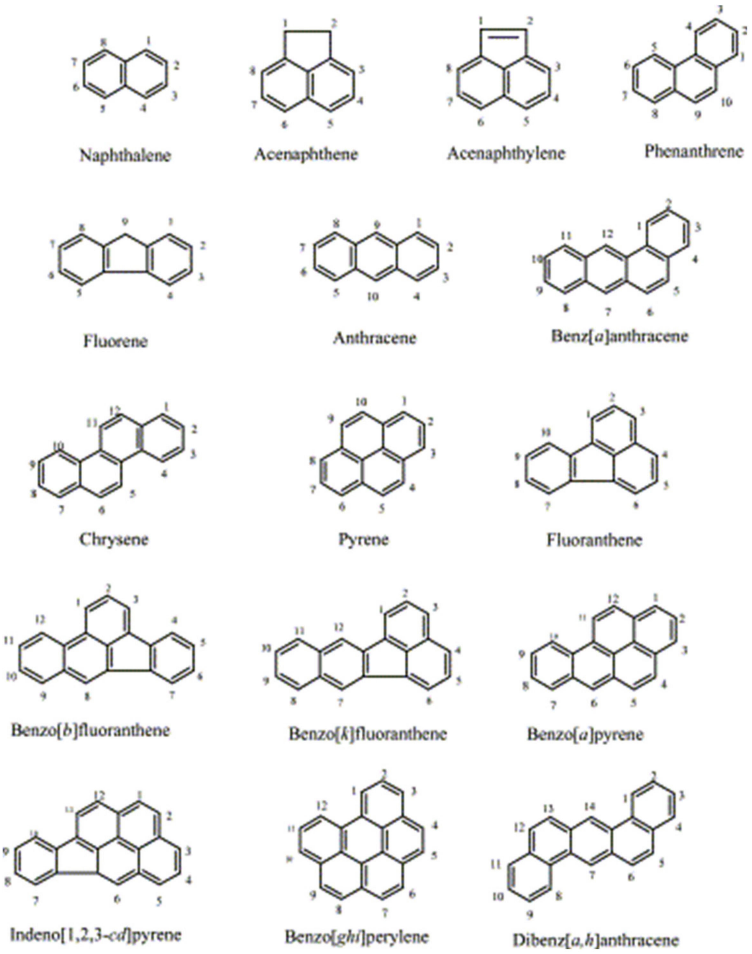

Figure 1. Structure formulas of the 16 analyzed PAHs (polycyclic aromatic hydrocarbons), adapted from [33,34].

This research is preliminary and aims to detect and quantify common PAHs in different, commercially available malts that can be used in different food industries (brewing, baking, etc.). All malts were subjected to different kilning temperatures, and our hypothesis is that they will display higher values of PAHs for higher temperatures. This is a good starting point for further investigations that could be used for setting the limits for PAH concentrations in cereals and malts intended for further processing in different food industries.

2. Materials and Methods

2.1. Sample Preparation and GC-MS Analysis

Malt samples were purchased from the local malting and brewing equipment store. Amber, black, pilsner and cara-120 malt were chosen for their different kilning temperatures. Table 2 shows the usual kilning temperatures for each malt.

Table 2. Kilning temperatures for analyzed malts.

Malt	Maximal Kilning Temperature (°C)	Source
Amber	100–150	[35]
Black	250	[36,37]
Pilsner	70–80	[38]
Cara-120	100	[39]

To prepare the PAH standards, a PAH mix of 16 polycyclic aromatic hydrocarbons (Ultra Scientific, North Kingstown, RI, USA) was used (500 ± 0.2 μg/mL). Samples were prepared using multiresidue preparations (QuEChERS), according to the Association of Analytical Communities' (AOAC) official method 2007.01 for extraction and clean up, described by [40]. The GC-MS parameters were adjusted, as described by Mastanjević et al. [9]. The PAHs determination method was modified, according to the accredited method ISO 17025.

Validation of precision, reproducibility, accuracy, linearity, LOQ (limit of quantification), LOD (limit of detection), and uncertainty is shown in Table S1. The method precision was evaluated, as described by Mastanjević et al. [9].

To eliminate the matrix interference, a calibration through a blank sample was performed, as well. The retention times of the peaks and target ions, obtained from the standard solution of PAHs, served as a base point for the PAH determination in samples.

2.2. Statistical Analysis

Data analysis was done using the analysis of variance (ANOVA) and Fisher's least significant difference test (LSD), with a statistical significance set at $p < 0.05$. Statistical analysis was carried out using Statistica 13.1. (TIBCO Software Inc., Palo Alto, CA, USA).

3. Results and Discussion

Foods, such as barley and malt, are processed using fossil fuels and, therefore, can contain hydrocarbons. Two procedures, direct and indirect firing, are used for the kilning of green malt; the direct heating of grains with fossil fuels leads to higher levels of PAH contamination [31]. Hutt et al. [41] reported that all combustion gases produced pass through the grain column during the process of the direct kiln-drying of grains, such as barley.

The conducted research considered different commercially available malts as a source of PAHs. All samples were chosen based on the kilning temperature, with the assumption that PAHs concentration will increase in samples kilned at higher temperatures. The obtained results, displayed in Table 3, showed that in all samples Flu, Ant, and BaA were detected. The highest concentration of BaA, reaching over 737 μg/kg, was quantified in black malt, while the lowest was quantified in amber malt (60.53 μg/kg). Pilsner and cara-120 malts showed high values for BaA, 134.26 and 210.7 μg/kg, respectively. All samples were statistically different.

Ant displayed the highest concentration in amber malt, while black and cara-120 malts showed similar values for this PAH (47.91 and 47.75 μg/kg), with no noted significant, statistical difference. Similar research was conducted by [29], but the values for caramel malt were significantly lower, 0.08 μg/kg, if we consider BaA concentrations.

Flu was quantified in highest concentration in black malt, going over 88 μg/kg. All malt samples showed significant statistical differences for this PAH, with the lowest concentration detected in cara-120 malt (7.68 μg/kg). However, amber and pilsner malt also displayed relatively high concentrations of Flu (18.17 and 14.61 μg/kg).

The highest concentration of Pyr was quantified in black malt, 7.17 μg/kg. Ane concentration was slightly higher in pilsner malt (0.21 μg/kg), in comparison to amber (0.18 μg/kg). Flt was quantified only in amber malt, with 10.44 μg/kg.

The sum of PAH4 was highest for black malt, resulting in 737.57 μg/kg. All other malts showed extremely high values for sum of PAH4, with the lowest being quantified in amber malt, 60.53 μg/kg. It should be noted that, in this case, the sum of PAH4 is the same as the values for BaA, since BaP, BbF, and Chry were not quantified in any of the samples. This is not in accordance with the research conducted by [30], where they did not detect PAh4 in regular malt samples, and only small amounts were reported in some of the smoked malt samples.

The sum of PAH16 was highest for black malt, with 881.52 μg/kg, but all other samples also showed high levels of PAH16.

Table 3. Concentrations (μg/kg) of PAHs (polycyclic aromatic hydrocarbons) in analyzed malt samples.

Malt Sample	**Amber**	**Black**	**Pilsner**	**Cara-120**
PAH	μg/kg			
Nap	<LOQ	<LOQ	<LOQ	<LOQ
Anl	$8.43^{a} \pm 3.64$	<LOQ	<LOQ	<LOQ
Ane	$0.18^{b} \pm 1.15$	<LOQ	$0.21^{a} \pm 0.54$	<LOQ
Flu	$18.17^{b} \pm 2.77$	$88.86^{a} \pm 0.20$	$14.61^{c} \pm 0.98$	$7.68^{d} \pm 2.19$
Ant	$81.24^{a} \pm 5.61$	$47.91^{c} \pm 2.15$	$49.87^{c} \pm 1.15$	$47.75^{c} \pm 0.84$
Phen	<LOQ	<LOQ	<LOQ	<LOQ
Flt	$10.44^{a} \pm 0.14$	<LOQ	<LOQ	<LOQ
BaA	$60.53^{b} \pm 0.03$	$737.57^{a} \pm 0.03$	$134.26^{c} \pm 0.03$	$210.7^{b} \pm 0.81$
Pyr	<LOQ	$7.17^{a} \pm 0.03$	$3.87^{b} \pm 0.03$	<LOQ
Chry	<LOQ	<LOQ	<LOQ	<LOQ
BbF	<LOQ	<LOQ	<LOQ	<LOQ
BkF	<LOQ	<LOQ	<LOQ	<LOQ
BaP	<LOQ	<LOQ	<LOQ	<LOQ
DahA	<LOQ	<LOQ	<LOQ	<LOQ
BghiP	<LOQ	<LOQ	<LOQ	<LOQ
Inp	<LOQ	<LOQ	<LOQ	<LOQ
∑PAH4	$60.53^{b} \pm 0.03$	$737.57^{a} \pm 0.03$	$134.26^{c} \pm 0.03$	$210.7^{b} \pm 0.81$
∑PAH16	$178.99^{d} \pm 11.10$	$881.52^{a} \pm 5.34$	$202.83^{c} \pm 3.48$	$266.21^{b} \pm 3.26$

[a–d] Means ± standard deviation within rows with different superscripts are significantly different ($p < 0.05$); LOQ—limit of quantification. ∑PAH4: BaA, Chry, BbF, and BaP. ∑PAH16: Nap, Anl, Ane, Flu, Ant, Phen, Flt, BaA, Pyr, Chry, BbF, BkF, BaP, DahA, BghiP, and InP.

In general, amber malt showed that it can contain Anl, Ane, Flu, Ant, Flt, and BaA, while cara-120 was designated only for Flu, Ant, and BaA.

Even though it was expected that pilsner malt, subjected to lowed temperatures during kilning, would result with lower PAH values, in this research, that was not the case. Namely, amber malt showed lower values for BaA and PAH4. This could be due to the fact that amber malt undergoes a very brief heating step at the end, just to gain color, while pilsner malt is subjected to kilning temperatures for a longer period of time (more than 24 h) [35,38].

4. Conclusions

The results of this research indicate that there is a need for the further, deeper, and broader research of PAHs in malt. The occurrence of some of the PAHs in malt is considerably dangerous and should gain the attention of the scientific and legislative audience. If we apply the EFSA set boundaries for PAH4 in processed cereal-based foods products, these results exceed the set values, by far. Obviously, the biggest issue is BaA, present in all samples, in significant amounts (60 μg/kg in amber and a whopping 737 μg/kg in black malt). This resulted in extremely high PAH4 levels in all samples, as well. Since the EFSA set BaA as one of the indicators for PAH4 toxicity, this calls for attention and further studies to be conducted, in order to present more reliable data about this subject. PAHs in beer should also be considered, but PAHs in barley are also important. A follow up on PAHs from barley, malt, and beer is eminent for further actions. These results could aid the inclusion of PAH concentrations in cereals, cereal-based foods, and beverages (which are, so far, not recognized by the European food safety authorities).

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/beverages7030058/s1, Table S1: The average values for precision, reproducibility, accuracy, linearity, LOQ and LOD for PAH method validation.

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