The effect of various flavorings on PAHs level in the shell and kernel of roasted sunflower seeds

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Research Article

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Abstract

Background: Amount of PAHs can be reduced by various methods, such as food additives. The effects of different flavors were investigated on the formation of carcinogenic and non-carcinogenic poly aromatic hydrocarbons (PAHs) in the shell and kernel of roasted sunflower seeds.

Methods: Samples were prepared in the shell and kernel of 10 types of sunflowers with flavors of lemon, golpar (hogweed), salt, ketchup and raw samples was performed with conventional solvent extraction by Soxhlet. Measuring and determining the amount of PAHs was analyzed by GC/MS.

Results: The PAHs of sunflower seeds were in the range of 0.4_3.2 ppm. The lowest amount was related to hogweed kernel and the highest amount was related to lemon shell.

Conclusion: Roasting process could result in contamination of sunflower seeds, especially the shell of seeds. Also, various flavors such as hogweed due to their antioxidant properties can affect the amount of PAHs. In addition, the excessive reduction of pH due to the oxidation of fats does not have a decreasing effect on the amount of PAHs. As a result, this amount was observed in lemon juice more than other flavors in shell.

1. Introduction

PAHs are a category of over one hundred chemicals that are found naturally in organic materials like coal, oil, fossil fuel, wood, garbage, and tobacco. Based on the number of carbon units, they are classified into two categories: “light PAHs” and “heavy PAHs”, PAHs with 5 or more carbon atoms are in the heavy category and PAHs with less than 5 carbon units are in the light category. The heavy PAHs are more stable and more toxic than the light ones (McGuire 2014, Ewa and Danuta 2017). Human exposure to PAHs is through air, water and food, but the highest exposure is observed through food (Kalteh, Rastkari et al. 2020, Akbari-Adergani, Mahmood-Babooi et al. 2021). Food contaminated with PAHs largely arises from production practices(Wenzl, Simon et al. 2006, Lee, Jeong et al. 2018). Unprocessed plant foods may be contaminated with PAHs through contaminated air and soil (5).

Considering the confirmed risk of PAHs to human health, measuring these components in environmental media and foodstuffs is of deep(Moazzen, Ahmadkhaniha et al. 2013). The International Agency for Research on Cancer (IARC, 2010) has classified benzo[a]pyrene as a group 1 carcinogen. While benz[a] anthracene (BaA), chrysene (Cry), benzo[k] fluoranthene (BkF) and benzo[b] fluoranthene (BbF) are classified in group 2B (probably carcinogenic to humans). The European Union(EU) Commission has set various maximum levels for PAHs in certain foodstuffs, with a focus on foods that contain fats and oils or where the food is smoked. According to these regulations, the maximum accepted level of benzo[a]pyrene is 5 μg/kg in smoked meats. (Rose, Holland et al. 2015). Due to the large family of PAHs, several key compounds have been introduced to indicate the presence of PAHs in food. The first PAH proposed as an indicator was Benzo[a]pyrene (BaP). But in 2008, the European Food Safety Authority (EFSA) declared that BaP alone cannot be a good indicator of the occurrence and carcinogenic effect of PAHs in food and covers all PAHs in food. So Eight compounds were selected as the most suitable indicators [PAH8; chrysene, benzo[k]fluoranthene, dibenz [a,h] anthracene, benzo[a] anthracene (BaA), BaP, indeno[1,2,3-pyrene (IP), benzo[b]fluoranthene (BbF), and benzo [ghi] perylene (BghiP)(EFSA, 2008).

Roasted sunflower seeds can be a source of PAHs due to roasting and the presence of unsaturated fats(Guo, Na Jom et al. 2019).Consumption of these nuts is very popular in Iran. Roasting involves a number of physicochemical changes including dehydration and chemical reactions(Alamprese, Ratti et al. 2009). Among the food preparation processes, smoking and grilling more than other processes lead to an increase in the amount of PAHs. Therefore, the source of PAH in roasted sunflower seeds is the contamination of raw sunflower seeds with environmental contaminants and contaminants produced during the roasting process(Akbari-Adergani, Khaki et al. 2017). PAH levels in smoked or grilled products can be reduced in a number of ways. These methods include: placing the product at a greater distance from the heat source, reducing the duration and temperature of heat treatment, selecting products with lower fat content, proper packaging and the use of additives that reduce the formation of PAH(Zeng, Li et al. 2021). In several studies, the effect of food additives and the formation of PAHs in grilled meats was investigated. The results of these studies show that the physicochemical properties of food affect the amount of PAH in food products (Farhadian, Jinap et al. 2012, Li, Cai et al. 2021, Onopiuk, Kołodziejczak et al. 2021). These properties include pH, moisture content, antioxidant activity, fatty acid composition, etc .Different flavorings can affect these properties and thus the production of PAHs.

Considering the relatively high consumption of sunflower seeds in the Iranian basket, suitable solution should be adopted to reduce PAHs in roasted sunflower seeds. The aim of this study was to investigate the effect of different flavors on the amount of PAHs in roasted sunflower seeds.

2. Materials And Methods

Sampling

Samples of sunflower seeds with different flavors of hogweed, ketchup, lemon, salt as well as raw samples were collected from one of the reputable factories in Tehran and transferred to Tehran University of Medical Sciences for preparation. Their shell and kernal were separated.

Sample preparation

The shell and kernel of samples were powdered by an electric grinder. In the next step, 50 g of the sample was poured into a 500 ml round bottom Erlenmeyer flask. Then its volume was increased to 100 ml, with ethanol and water at a ratio of 1:9. 8.4 g of potassium hydroxide and 0.5 ml of standard xylene internal solution (at a concentration of 1 μg / ml in methanol) was added. This solution was refluxed for 3 h. Then, the prepared shell sample was filtered by porous filter No. 3 and the kernel sample was filtered by Buchner filter. In order to wash the sample, 20 ml of methanol and water in a ratio of 1: 9 was added. Then, this solution with 50 ml of isooctane in a decanter and shaken each time for 5 minutes, and collected the isooctane phase in a container and the resulting extract was washed with 100 ml of methanol and water in a ratio of 1: 1. Isooctane solvent was extracted twice and each time with 50 ml in decanter for 5 minutes. To the isooctane phase, 10 g of active sodium sulfate powder was added, shaken for 1 minute by a shaker, then filtered through a sieve, and finally the isooctane phase was evaporated in rotary to a volume of 2 ml at 55 ° C.

GC–MS Analysis

Separation, determination and identification of the target analytes were performed using an Agilent 7890A gas chromatograph was equipped with a 5975-mass selective detector (MSD, Agilent Technologies) and fitted with Restek Rxi®-5MS fused-silica capillary column 5% Phenyl Methyl Silox (60 m × 0.32 mm i.d. and 0.25 μm film thickness). Carrier gas, Helium (purity 99.999%) was set at flow rate of 1.0 mL min^{-1}.

During the whole analysis, the injector was operated in the splitless mode with an injector temperature of 290 °C. The oven temperature was initially set at 70 °C (2 min hold), followed by a temperature ramp of 20 °C min^{-1} to 220

°C, and held for 2 min, then increased to 295 °C at a rate of 5 °C min^{-1}, and eventually held for 8 min, and the solvent delayed for 4 min, for a total run time of 34.5 min. The MSD was operated in electron impact (EI) mode at 70 eV with an ion source temperature of 230 °C. MSD transfer line and quadrupole temperatures were 290 and 150°C, respectively. The sample extracts and standards were injected (2 µL) into the GC-MS. The MSD system was routinely programmed in selective ion monitoring (SIM) mode. The identification of PAHs in the standard mixture was done by comparing the chromatography based on the retention time(Fig 1).

3. Results

3.1. Analytical performance

The Limit of detections (LODs) for each PAHs were obtained in the range of 0.5 µg kg^{-1} practically based on signal-to-noise ratio of 3. Limit of quantifications (LOQs) was 0.8 µg Kg^{-1}. Linear dynamic ranges (LDRs) were 0.8 to 400 µgKg^{-1} with coefficient of determination (r^2) from 0.9898 to 0.9996. Quantification and identification ions (m/z) of 16 PAHs were shown at Table 1. Confirmation of the PAHs was established by the retention time and the presence of the target ions. The target ion abundances were determined by injection of PAHs standard under the same chromatographic condition but utilizing full-scan conditions with the mass/charge scan ranging from 40 to 550 m/z.

Table 1
Name of PAHs component and Instrumental analyses characteristics

No.	PAH	Abrev.	RT(min)	M/Z
1	Naphthalene	Nap	7.742	128, 129, 102
2	Acenaphthylene	Acp	9.799	152,153,151
3	Acenaphthene	Ace	10.015	153, 154, 76
4	Fluorene	Flu	10.773	166, 165, 82
5	Phenanthrene	Phe	12.451	178, 176, 76
6	Anthracene	Ant	12.559	178, 179, 79
7	Fluoranthene	Flt	15.590	202, 203, 101
8	Pyrene	Pyr	16.294	202, 203, 200
9	Benz[a]anthracene	BaA	20.840	228, 226, 114
10	Chrysene	Cry	21.003	228, 226, 229
11	Benzo(b)fluoranthene	BbF	25.225	252, 253, 126
12	Benzo[k]fluoranthene	BkF	25.333	252, 253, 113
13	Benzo[a]Pyrene	BaP	26.470	252, 253, 250
14	Indeno[1,2,3-cd]pyrene	InP	31.449	276, 277, 138
15	Dibenzo[a,h]anthracene	DBA	31.611	276, 277, 139
16	Benzo[ghi]perylene	BghiP	32.802	276, 277, 138

RT, retention times of GC-MS chromatogram

M/Z, selected ions for target pesticides used as quantifier and qualifier respectively

3.2. The amount of PAHs in different type of sunflower seed

The amounts of PAHs in the shell and kernel of sunflower seeds with different flavors of ketchup, Hogweed (golpar), lemon, and salt and in raw samples were compared with each other (Fig. 1). The mean and standard deviation of these PAHs are shown in Table 2. The PAHs of sunflower seeds were in the range of 0.4_3.2ppm. The lowest amount was related to hogweed kernel and the highest amount was related to lemon shell. Also the raw samples did not have PAHs. A significant difference was seen between the PAH concentrations in the roasted shell and roasted kernel of the sunflower seeds. In this case, Mann-Whitney test was used for comparison. A significant difference was observed between lemon shell and lemon kernal) P = 0.02(. The large amount in the shell is probably due to the direct contact of the shell with heat(Akbari-Adergani, Khaki et al. 2017).

PAH levels included PAH8 (B[a]A, CHR, B[b]F, B[k]F, B[a]P, D[a,h]A, I[c,d]P, and B[g,h,i]P) was calculated for all samples and was ND. Low molecular weight PAHs were also measured, their average is shown in Table 3. For high molecular weight PAH, only fluoranthene was observed, the rest were ND. It is important to note that the heavier compounds, in addition to being more stable, have a higher lipophilic character, a characteristic that facilitates their absorption by the body(Rey-Salgueiro, Martínez-Carballo et al. 2009).

Table 2
Average concentrations of total PAHs (ppm) in the shell and kernel of roasted and raw sunflower seeds

Samples	Average and Standard deviation
Raw kernel	ND
Raw shell	ND
hogweed kernel	0.4 ± 0
hogweed shell	2.2 ± 1
Lemon kernels	0.68 ± 0.12
Lemon shell	3.2 ± 0.4
Ketchup kernel	1.8 ± 0.2
Ketchup shell	1.48 ± 1.4
Salt kernel	1.2 ± 0.8
Salt shell	2 ± 1.2

Table 3
Average concentrations of low molecular weight of PAHs (ppm) in the shell and kernel of roasted and raw sunflower seeds

samples	Salt shell	Salt kernel	Ketchup shell	Ketchup kernel	Lemon shell	Lemon kernels	hogweed shell	hogweed kernel	Raw shell	Raw kernel
Average of LMW*	1.8 ± 1	0.6 ± 0.5	1.28 ± .48	1.6 ± 0	2.8 ± 0.8	0.68 ± 0.12	2.2 ± 1	0.2 ± 0.18	ND	ND
*LMW: low molecular weight										

4. Discussion

This study showed the role of food additives on the amount of PAHs in sunflower seeds. The roasting process produces PAHs in sunflower seeds. Unsaturated fatty acids, that is present in sunflower seeds in large quantities, such as linoleic acid and linolenic acid, can easily generate cyclic monomers through polymerization, and finally form PAHs precursors containing benzene rings (Nie, Cai et al. 2019, Nie, Cai et al. 2020). In this study, the lowest value was seen in hogweed samples (Table 2). Hogweed contains flavonoids, coumarin, polyphenols. All three of these compounds have antioxidant properties. Natural antioxidant compounds are safe compounds(Sadighara, Godarzi et al. 2016). The presence of antioxidants is effective in determining the amount of PAHs in heated or smoked products(Viegas, Amaro et al. 2012). The mechanism of action is that antioxidants can act as radical scavengers and suppressants for PAH formation. Also, since lipid is an important precursor in the production of PAHs, antioxidants can be used to prevent lipid oxidation to reduce the formation of PAHs (Gong, Zhao et al.

2018). In a study, the effects of different antioxidants on the formation of PAH in meat samples were investigated. After determining the most effective concentration, individual antioxidants (BHT, BHA, α-tocopherol, EGCG and sesamol) were added to the meat. Total PAH in all meat samples decreased with added antioxidants(Min, Patra et al. 2018). The results presented by Wang et al. (2019) and Cordeiro et al. (2020) show that phenolic compounds inhibit PAHs in two ways: scavenging free radicals and preventing the formation of PAHs through more complex interactions such as binding to PAH intermediates, but this issue needs more research(Onopiuk, Kołodziejczak et al. 2021). Also, lycopene is a powerful antioxidant found in ketchup(Wu, Gao et al. 2021). In this study, there was no significant decrease in the amount of PAHs in the ketchup samples. Lycopene is a natural carotenoid that can reduce the toxicity of PAHs in the body of living organisms (Ma, Saad Eldin et al. 2019). Therefore, it does not have the ability to reduce PAHs in food environments. Although extensive research is needed in this regard.

In this study, the level of PAHs in the kernels of sunflower seed samples processed with lemon was also low (Table 2). Wongmaneepratip et al. (2017) in a study, found that a slight increase in the pH value can significantly increase the PAH content in grilled chicken (Wongmaneepratip and Vangnai 2017, Li, Cai et al. 2021).The addition of lemon juice lowers the pH and possibly affects the rate of the PAH formation reaction. Additionally, the sulfur dioxide compound in lemon juice may help reduce PAH by inhibiting Maillard reactions(Farhadian, Jinap et al. 2012, Zhang, Chen et al. 2021). During Maillard reactions, a reaction between an amino acid and a reducing sugar produces a Schiff base. This Schiff base itself is unstable and is easily oxidized and enters the production cycle of Amadori compounds. These compounds may undergo intricate reactions (pyrolysis and polymerization) to form PAHs such as pyrene and BaA(Britt, Buchanan et al. 2004, Lund and Ray 2017). In this way, reducing the Maillard reaction helps to lower the PAHs. Similarly, Farhadian et al.(2012)explained that the addition of acidic substances, rich in polyphenols, i.e. lemon juice or tamarind to marinade treatments, reduced PAH formation in grilled beef(Farhadian, Jinap et al. 2012). On the other hand, in the article by Cordeiro, T., et al. (2020), excessive reduction of pH did not prevent PAHs production(Cordeiro, Viegas et al. 2020). Perhaps the reason is that acidic marinades (acetic acid, citric acid, lactic acid, etc.) greatly affect the structure of cooked meat (Önenç, Serdaroğlu et al. 2004, Sharedeh, Gatellier et al. 2015) and acid treatments reduce water holding capacity and increase fat and protein oxidation in meat. All these microstructural changes may cause non-inhibition of PAH at very low pH.

It has been reported that PAH formation is related to water content, and PAH content was higher in moist meat models compared to dry samples(Li, Cai et al. 2021). Many previous studies have shown that sodium can cause changes in, water content, pH, or fatty acid composition and reduces the Millard reaction, which indirectly cause changes in PAHs value (Guàrdia, Guerrero et al. 2008, Li, Cai et al. 2021). Furthermore, salts (including, calcium chloride, magnesium chloride, potassium lactate, and calcium lactate), that can reduce the pH of meat products can prevent the production of PAHs(Li, Cai et al. 2021). In this study, salt also had a positive effect on reducing the amount of PAHs with low molecular weight in sunflower seed kernels (Table 3).

In addition to the flavorings mentioned, reported meat flavorings such as onion, paprika, red pepper, black pepper powder, garlic and ginger prevent the formation of PAHs. which can be caused by their antioxidant activity(Lu, Kuhnle et al. 2018).

The amount of PAHs in raw sunflower seeds usually indicates the amount of pollution in the air, soil and water. Because PAHs was not found in raw sunflower seeds in the present study, polycyclic aromatic hydrocarbons directly contaminate sunflower seeds as a result of the roasting process (Akbari-Adergani, Khaki et al. 2017). Therefore, PAH formation is strongly affected by the cooking method, alternative cooking methods in the initial preparation stage can help to effectively reduce PAH levels. Also, according to the studies, the amount of PAH caused by cooking decreases at a lower cooking temperature.

Conclusion

The amount of PAHs in sunflower seeds can be affected by different flavors. This amount can be increased or decreased according to flavoring properties such as flavoring antioxidant properties or changing the pH of the environment. Among different flavorings, the amount of PAHs in a sample of hogweed was the lowest. Also, since the raw samples did not have PAHs, the roasting process can lead to contamination of the seeds, especially the seeds shell. In this study, the amount of total PAHs in all flavors was higher in the shell, except for the ketchup sample, the amount of PAHs in kernel was higher, which was not statistically significant. In this study, PAHs with high molecular weight were not observed in the samples of roasted sunflower seeds. Therefore, this product will probably not be considered a threat due to the risk of carcinogenesis.

Declarations

Conflict of interest

The authors of this article declare that they have no conflict of interests.

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Author contribution statement

The analysis of heavy metals in samples was conducted by Parisa Shavali-Gilani, najmeh yazdanfar. The analysis of data was performed by Parisa Sadighara , Ebrahim Molaee- Aghaee The manuscript was written by parisa shavali-gilani, Parisa Sadighara and Gholamreza Jahed -Khaniki.

Data availability

The datasets generated and analysed during the current study are presented in the manuscript

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Figures

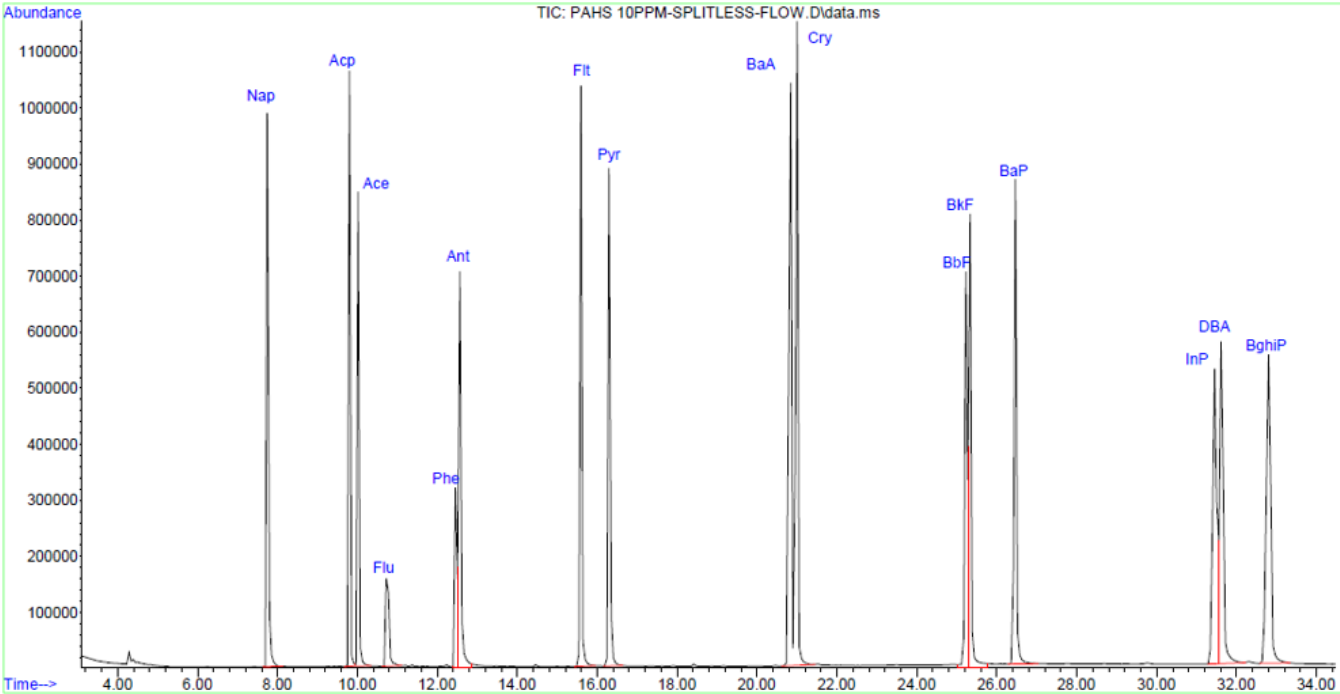

Figure 1

Chromatogram of standard mixture of 10ppm polycyclic aromatic hydrocarbons (PAHs)

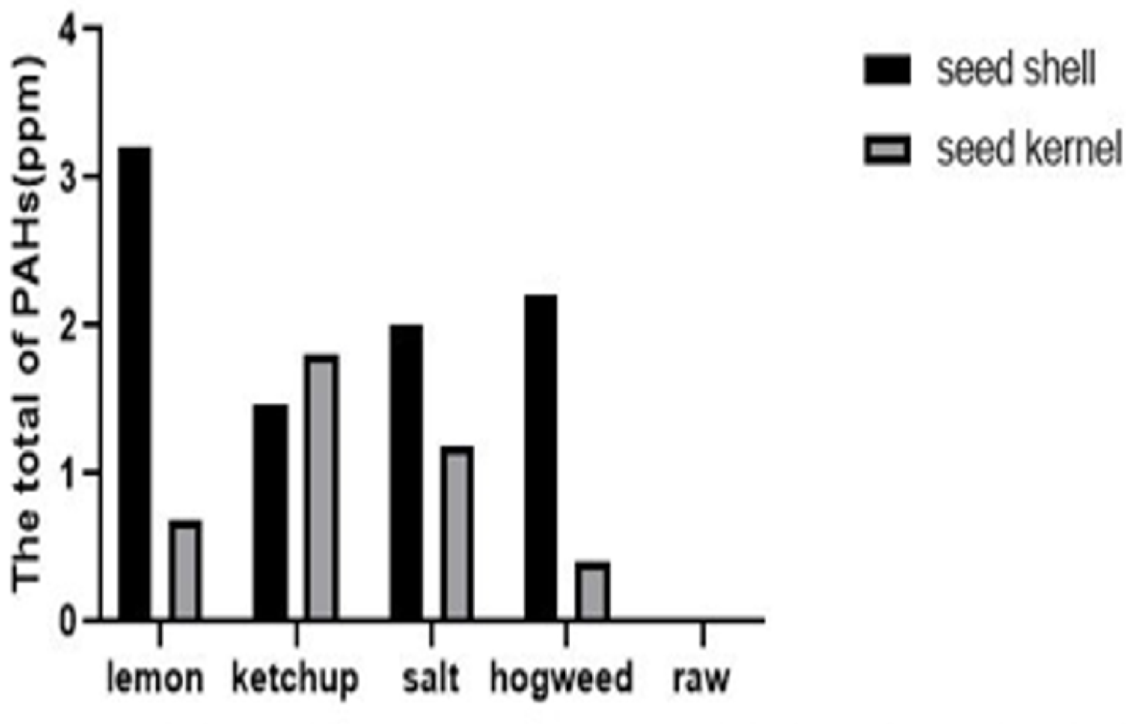

Figure 2

1. Comparison of PAHs between different flavors of raw and roasted sunflower seeds