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Comparison of different analytical methods on determination of polycyclic aromatic hydrocarbons (PAHs) in ketchup-flavored sunflower seeds

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ABSTRACT

It is well-established that the presence of polycyclic aromatic hydrocarbons (PAH) in food can trigger the activation of carcinogenic agents, leading to genotoxic and mutagenic effects. Several analytical techniques have been employed to determine PAH levels in food. The effectiveness of extracting PAH concentrations from food samples relies on the specific extraction methods. Also, the selection of the extraction method is influenced by the characteristics of the food. The main purpose of this study was to compare the efficiency of two extraction methods for the analysis of polyaromatic hydrocarbons (PAHs) in ketchup-flavored sunflower seeds. The separation of PAHs was carried out using the soxhlet extraction method and sonication extraction method. The efficiencies of extraction were determined through a thorough analysis using GC/MS. In this study, there was no significant difference between soxhlet and sonicate extraction methods in extracting a total 16 PAH ($p<0.05$).

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1. Introduction

Today, one of the most important issues is providing healthy and pollution-free food for the world's population.

Food can be tainted by physical, chemical, or microbial contaminants, which may arise from various factors during its production, storage, transportation, or processing (1). These pollutants can have various adverse effects on human health including acute or

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chronic poisoning. Therefore, identification, detection, and measurement of of the amount of these pollutants in food is very important for human health and require research and analytical methods (2).

Due to the increasing concentration of PAHs in the environment, one of the serious concerns of the world today is the contamination of food with these pollutants. PAHs are organic compounds that consist of two or more benzene rings in various forms. There are two categories of polycyclic aromatic hydrocarbons (PAHs), with one being light PAHs containing two to four fused rings and the other being heavy PAHs containing more than four fused rings. Heavy PAHs, which are more stable and toxic, are characterized by their greater number of rings (3,4).

PAHs as environmental pollutants are mainly produced from the incomplete combustion of organic materials, which are caused by anthropological activities or sometimes natural phenomena such as fires, volcanic activity, oil disposal, etc. Therefore, they are found in water, air and soil (4,5). Furthermore, these contaminants have the potential to infiltrate food through various means, such as during the different stages of food preparation (e.g., roasting, barbecuing, smoking, baking, frying, and drying), food packaging processes, and through the absorption of contaminated air (2, 4-7). In addition to that, there are other factors that affect the contamination of food with PAH compounds, which include fuel (wood, coal, gas, electric power), temperature, cooking time, food fat, and the amount of direct contact between flame and food (7).

So far, over 100 polycyclic aromatic hydrocarbon (PAH) compounds have been recognized, with the majority being produced through pyrolytic reactions. Among them, 16 PAHs have been identified as major contaminants of food sources by the United States Environmental Protection Agency (EPA), including naphthalene (Nap), fluorine (Flu), acenaphthylene (Ace), anthracene(Ant), fluoranthene (Flt), benz[a]anthracene (BaA), pyrene (Pyr), chrysene (Cry), acenaphthene (Acp), benzo[b] fluoranthene (BbF), benzo[g, h, i] perylene (BghiP), phenanthrene (Pha),benzo[k]fluoranthene(BkF), benzo[a] pyren (BaP), indeno [1,2,3-cd] pyrene (InP), and dibenz[a, h] anthracene (DBahA) (8,9). Also, among these 16 compounds, Benzo[a]pyrene (BaP) has been categorized as category 2A by the International Agency for Research on Cancer (IARC) as a compound with the possibility of carcinogenesis in humans by food contaminated with PAHs (10). According to the calcification of the World Health Organization, these compounds can enter the body through respiratory, gastrointestinal, and dermal routes and after rapid absorption, they are distributed in the body and can have mutagenic and carcinogenic effects for humans. In addition to carcinogenicity, PAHs can also cause possible genotoxic effects and Immunosuppressive effects (11). And increasing the lipophilic properties of these compounds increases their absorption in the gastrointestinal tract (12). It has been observed that in non-smokers and non-occupational people, consumption of contaminated food and water is the main source of PAHs entering the body (13, 14). Studies have reported a positive association that there is a positive association between the consumption of food contaminated with PAHs and complications such as gastric cancer, lung problems, and cytogenetic and

biochemical changes (15). Due to the potential that PAHs have for toxic effects and cancer in humans through food, it is very important to monitor and control their concentration in food products (4). numerous organizations evaluate the levels of these compounds, including the International Program on Chemical Safety (IPCS), the Scientific Committee on Food (SCF), the United States Environmental Protection Agency (US EPA), the European Food Safety Authority (EFSA), the WHO International Agency for Research on Cancer (IARC), and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (16).

Sunflower seeds are a highly popular food item worldwide and are particularly favored among the population of Iran, especially during festive occasions, social gatherings, and evening events. Roasted and flavored sunflower seeds are more popular and many people prefer to consume sunflower seeds flavored with ketchup. These may absorb PAH compounds during transport, heating or packaging (17). The European Union has not established a specific threshold for aromatic polyhydrocarbon compounds in sunflower seeds. However, given the considerable consumption of sunflower seeds, it is crucial to assess the levels of PAH compounds present in them(1). Various methods have been studied to extract and evaluate the amount of these compounds in food. In this study, two extraction methods with Soxhlet and sonication were compared. GC/MS was used for analysis.

2. Materials and Methods

2.1. Reagents

PAH mix standards containing sixteen mentioned PAHs were obtained from Supelco Company (Bellefonte, PA, USA). The standard solution contained PAHs prepared in dichloromethane. Xylene was used as the internal standard (I.S.) (1 µg/mL in methanol). Sodium sulfate, Na_2SO_4), potassium hydroxide (KOH), isooctane, methanol, and silica gel were bought from Merck company (Germany). Ethanol was purchased from Dr. Mojalli Laboratories Industries (Iran). Filter paper purchased from Whatman company (England).

2.2. Sample preparation

Ketchup-flavored sunflower seeds were collected from the production factory. At First, the shells and kernels were separated, and they were powdered separately using an industrial mill.

2.3. Soxhlet extraction method

50 g of the samples were placed in a 500 mL Erlenmeyer flask. The volume was then adjusted to 100 mL with a mixture of ethanol and water in a 1:9 ratio. Subsequently, 8.4 g of potassium hydroxide and 0.5 mL of xylene internal standard solution (1 µg/mL in methanol) were added. The solution was refluxed for 3 h. The prepared shell sample was filtered using a No. 3 porous filter, while the kernel sample was filtered using a Büchner funnel. To wash the sample, a mixture of 20 mL of methanol and water in a 1:9 ratio was added, followed by extraction with 50 mL of isooctane. Each extraction involved shaking for 5 min, and the resulting isooctane phase was collected and washed with a mixture of 100 mL of methanol and water in a 1:1 ratio. The isooctane solvent was extracted twice with 50 mL each time in the decanter for 5 min. Ten grams of active sodium sulfate powder was added to the isooctane phase and shaken for 1 min with a shaker. It was then

filtered through a strainer and finally, the isooctane phase was evaporated in a rotary up to a volume of 2 mL at a temperature of 55 ℃ (Fig. 1). With this method, the sample was prepared for instrumental analysis.

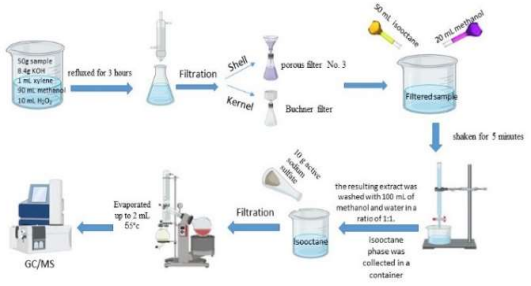

Figure 1. Soxhlet extraction method

2.4. Sonication extraction method

The second portion of each sample was obtained using the sonication technique. The samples were placed in an ultrasonic bath and extracted three times for 30 min with 150 mL of methanol during the initial extraction phase. This was followed by two extractions for 30 min each, using 20 mL of 0.7 M potassium hydroxide solution and 30 mL of distilled water during the secondary phase extraction. The filtrates from the latter phase were then transferred to a separator funnel containing n-hexane, and a rotary evaporator was used for drying. The solution was then made up to volume with 0.5 mL of acetonitrile. and injected into the GC/MS device (Fig. 2).

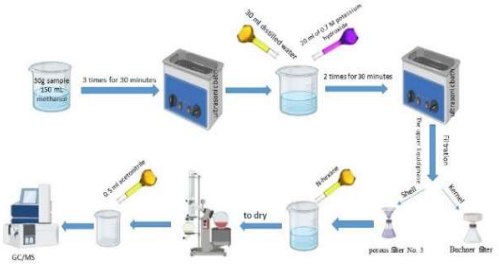

Figure 2. Sonication extraction method

2.5. GC/MS analysis

The instrumental analysis was performed using an Agilent 7890A gas chromatography apparatus which was outfitted with a 5975 mass selective detector manufactured by MSD Agilent Technologies. The Separation was achieved by a polydimethylsiloxane HP-5 (5−95%) capillary column. The column had dimensions of I.D 0.25 mm × 30 m, made of silica, with a film thickness of 0.25 micrometers and a quadrupole-type mass spectrometer. The injection temperature was maintained at 275°C. Helium was used as the carrier gas with a velocity of 1.4 mL/min. The oven temperature was initially set at 80°C for 2 min, then increased at a rate of 50°C/min to 230°C, followed by a gradual rise of 2°C/min to 260°C and a further increase of 8°C/min to reach 340°C, where it was held for 5 min. The entire duration of the run was 35 min. The peak spectra of each compound were analyzed by comparing them with the mass spectra of PAH standards. In this investigation, a total of 16 PAHs were examined, consisting of six low molecular weight PAHs (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, and anthracene) and ten high molecular weight PAHs (fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene, benzo[g,h,i]perylene and indeno[1,2,3-cd]pyrene) (Table 1). The analyses were performed in triplicate to ensure accuracy and reliability.

2.6. Statistical analysis

The data obtained from instrumental analysis were statistically analyzed using EXCEL and SPSS version 25 software. Analysis of variance (one-way) was used to

compare the average amount of PAHs between soxhlet extraction method and sonication extraction method.

3. Results

After GC/MS analysis, The PAHs identified by the sonication method in ketchup-flavored sunflower kernel were as follows: Nap, Flt, Pyr that the concentrations were 0.45±0.05, 0.75±0.2, and 0.75±0.2 µg/kg , respectively.Also the PAHs that were identified by soxhlet extraction methods in ketchup-flavored sunflower kernel were Acp, Phe, Flt, with concentrations of 0.65±0.1, 1±0.6, 0.45±0.05 µg/kg , respectively. Other PAHs such as Fluorene, Anthracene, Benzanthracene, Chrysene, Benzo(b)fluoranthene, Benzo[k]fluoranthene, Benzo[a]Pyrene, Indeno [1,2,3-cd]pyrene,

Table 1. Name of PAHs component, LOD, LOQ, and identification ions

No	PAHs	Abbreviations	RT(min)	m/z	LOD (µg/kg)	LOQ (µg/kg)	Recovery (%)
1	Naphthalene	Nap	7.742	128, 129, 102	3	9	89.27
2	Acenaphthylene	Acp	9.799	152,153,151	3	9	90.2
3	Acenaphthene	Ace	10.015	153, 154, 76	3	9	91.4
4	Fluorene	Flu	10.773	166, 165, 82	3	9	88.56
5	Phenanthrene	Phe	12.451	178, 176, 76	3	9	89.73
6	Anthracene	Ant	12.559	178, 179, 79	4	10	90.65
7	Fluoranthene	Flt	15.590	202, 203, 101	3	9	91.21
8	Pyrene	Pyr	16.294	202, 203, 200	3	9	89.83
9	Benz[a]anthracene	BaA	20.840	228, 226, 114	3	9	91.46
10	Chrysene	Cry	21.003	228, 226, 229	3	9	88.04
11	Benzo(b)fluoranthene	BbF	25.225	252, 253, 126	3	9	85.26
12	Benzo[k]fluoranthene	BkF	25.333	252, 253, 113	3	9	85.28
13	Benzo[a]pyrene	BaP	26.470	252, 253, 250	3	9	88.34
14	Indeno[1,2,3-cd]pyrene	InP	31.449	276, 277, 138	4	10	90.71
15	Dibenzo[a,h]anthracene	DBA	31.611	276, 277, 139	4	10	92.37
16	Benzo[ghi]perylene	BghiP	32.802	276, 277, 138	4	10	91.15

RT, retention times of GC-MS chromatogram; m/z, selected ions for target pesticides used as quantifier and qualifier respectively

Dbenzo[ghi]perylene, Dibenzo[a,h]anthracene, Benzo[ghi]perylene, were not detected by either method (Table 2). In this study, there was no significant difference between Soxhlet and Sonicate extraction methods in extracting a total of 16 PAH (Fig. 4). However, a significant difference was observed between these two extraction methods in the measurement of Phe ($p<0.01$) and Pyr ($p<0.05$) (Fig. 5).

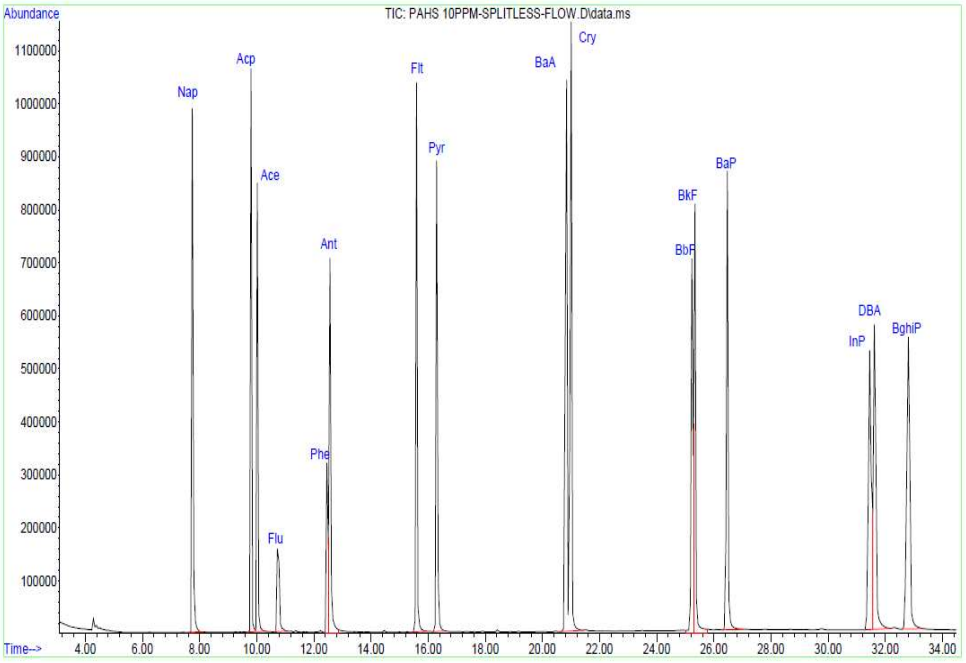

Figure 3. Chromatogram of standard mixture of 10 mg kg^{-1} polycyclic aromatic hydrocarbons (PAHs)

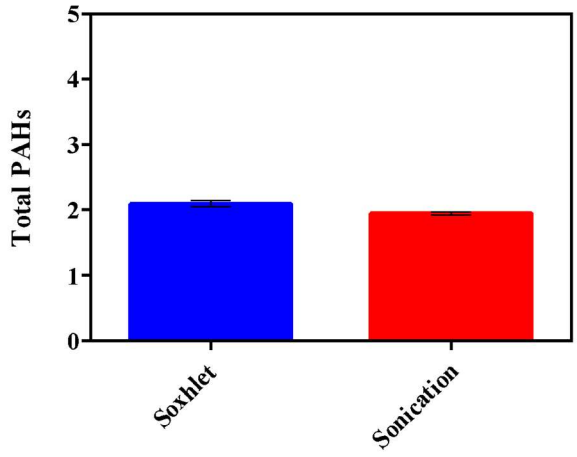

Figure 4. Comparison of 16 PAHs level (µg/kg) in ketchup-flavored sunflower kernel extracted by soxhlet and sonication methods

Table 2. Average PAHs in ketchup-flavored sunflower kernel by sonication and soxhlet extraction methods

No	PAHs	Abbreviations	Soxhlet extraction method (µg/kg)	Sonication extraction method (µg/kg)
1	Naphthalene	Nap	nd	0.45±0.05
2	Acenaphthylene	Acp	0.65±0.1	nd
3	Acenaphthene	Ace	nd	nd
4	Fluorene	Flu	nd	nd
5	Phenanthrene	Phe	1±0.6	nd
6	Anthracene	Ant	nd	nd
7	Fluoranthene	Flt	0.45±0.05	0.75±0.2
8	Pyrene	Pyr	nd	0.75±0.2
9	Benz[a]anthracene	BaA	nd	nd
10	Chrysene	Cry	nd	nd
11	Benzo(b)fluoranthene	BbF	nd	nd
12	Benzo[k]fluoranthene	BkF	nd	nd
13	Benzo[a]pyrene	BaP	nd	nd
14	Indeno[1,2,3-cd]pyrene	InP	nd	nd
15	Dibenzo[a,h]anthracene	DBA	nd	nd
16	Benzo[ghi]perylene	BghiP	nd	nd

nd: nondetectable

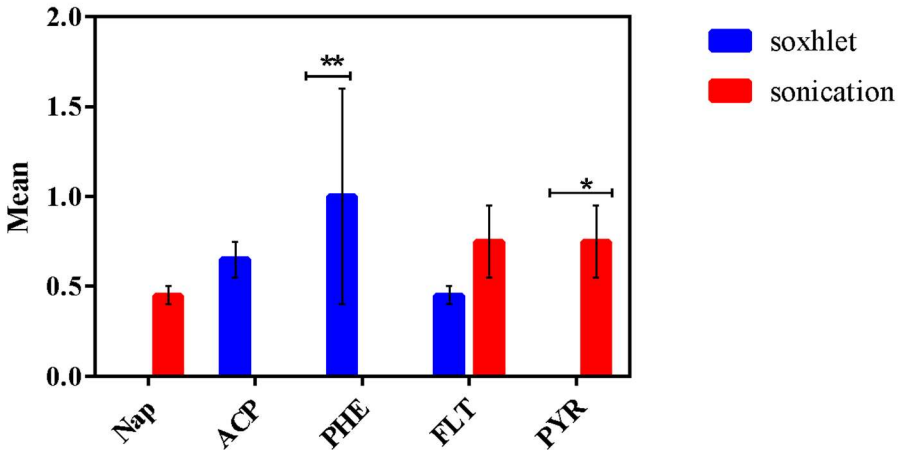

Figure 5. PAH levels (µg/kg) measured in ketchup-flavored sunflower seed kernels by soxhlet and sonication methods
* It is statistically significant

4. Discussion

Statistical analysis indicated no significant difference in the overall extraction of 16 PAHs between the two methods. However, significant differences were observed specifically for Phenanthrene ($p < 0.01$) and Pyrene ($p < 0.05$), suggesting that while both methods are effective for PAH extraction, they may yield different results for certain compounds. In a study of Kosar Mahmood-babooi et al. There was a significant difference between total PAHs mean values in tahdig by soxhlet and sonication methods ($p<0.05$) (18). Also in another study three different techniques, ultrasonic extraction, soxhlet extraction, and accelerated solvent extraction, were utilized to analyze polycyclic aromatic hydrocarbons in plant leaves. It was found that there were no notable differences in the extraction efficiencies of medium-molecular-weight and high-molecular-weight PAHs among these three methods (19). In another study, Soxhlet extraction demonstrated the capability to recover approximately 60 to 90% of the overall quantity of PAHs present in the pasture vegetation. In contrast, sonication exhibited lower efficacy, recovering only 10 to 50% of the PAHs. Also, it was observed that extraction efficiencies improved with higher molecular weight of the PAH compounds (17,20). The Soxhlet technique is renowned for its effectiveness in extracting polycyclic aromatic hydrocarbons (PAHs) from solid food materials, achieving an efficiency range of 84-100% for PAHs containing more than 4 rings. Nevertheless, it necessitates a substantial amount of solvents and elevated temperatures, potentially resulting in the loss of PAHs due to volatilization and/or oxidation of highly volatile compounds (21).

Both techniques may be successful in extracting PAHs, with the decision between them potentially influenced by variables such as the sample matrix, desired extraction efficiency, and equipment availability. Each method presents its strengths and weaknesses, and the choice of the most appropriate method should be guided by the specific needs of the analysis (22). Hence, careful consideration should be given to the selection of the extraction method and its parameters, taking into account the unique food matrix and the desired level of extraction efficiency for PAHs.

5. Conclusion

So far, there have been limited studies on related to the presence of PAH in sunflower seeds. In this study, two extraction methods, sonication, and Soxhlet, were compared to extract 16 PAH. There was no significant difference between two methods in extracting a total 16 PAH. But significant difference was observed between these two extraction methods in the measurement of PHE ($p <0.01$) and PYR ($p <0.05$). In this case, Soxhlet and Sonicate were more effective respectively. According to other studies, the selection of the extraction method is influenced by the characteristics of the food and target analyte.

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Conceptualization: Parisa Sadighara

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Funding acquisition: Nader Akbari

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Methodology: Parisa Shavali-Gilani

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Supervision: Parisa Sadighara

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Declaration of competing interest

The authors declare that they have no conflict of interest.

Data availability

Data will be made available on request.

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