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Determination of Changes in Polycyclic Aromatic Hydrocarbon (PAH) Levels in Hazelnut Oils After Storage

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Abstract: The oil of hazelnuts collected from a hazelnut garden in Piraziz District of Giresun Province in two different years, 2022 (called as S1) and 2023 (called as S2), was obtained and the polycyclic aromatic hydrocarbon (PAH) levels, known to have carcinogenic effects, were analyzed depending on the storage time and condition. The optimum parameters were maintained and analyzed by GC-MS. The highest total concentration of polycyclic aromatic hydrocarbons (Σ16PAHs) was 8.483 ± 0.032 ng/kg in sample S1 and 6.075 ± 0.024 ng/kg in sample S2. The value of naphthalene, the most abundant PAH analyte, is higher than that of the other analytes. The total amount of PAHs indicates that 92.35% of sample S1 and 87.82% of sample S2 are naphthalene. Notably, benzo[a]pyrene (BaP), a known carcinogen, was not detected in either sample.

Keywords: GC-MS, Hazelnut oils, Polycyclic aromatic hydrocarbons (PAHs), SPE sample preparation.

Depolama Sonrası Fındık Yağlarındaki Polisiklik Aromatik Hidrokarbon (PAHs) Düzeylerindeki Değişimlerin Belirlenmesi

***Sorumlu yazar:**
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Öz: Giresun İli Piraziz İlçesindeki bir fındık bahçesinden; 2022 (S1 olarak kodlanan) ve 2023 (S2 olarak kodlanan) iki farklı yılda toplanan fındıkların yağı elde edilmiş ve bekleme süresine ve koşuluna bağlı olarak, kansorejen etkisi bilinen polisiklik aromatik hidrokarbon (PAH) değişimleri ölçülmek istenmiştir. Optimum parametreler sağlanarak GC-MS ile analizler gerçekleşmiştir. Polisiklik aromatik hidrokarbonların (Σ16PAH) en yüksek toplam konsantrasyonu S1 örneğinde 8,483 ± 0,032 ng/kg ve S2 örneğinde 6,075 ± 0,024 ng/kg olarak bulunmuştur. PAH standart karışımı içerisinde, en fazla miktardaki analit olan naftalinin bulunma miktarı diğerlerinden daha yüksektir. Toplam PAH miktarı, S1 örneğinin %92,35'inin ve S2 örneğinin %87,82'sinin naftalin olduğunu göstermektedir. Bilinen bir kanserojen olan benzo[a]piren (BaP) her iki örnekte de tespit edilmemiştir.

Anahtar kelimeler: GC–MS, Fındık yağları, Polisiklik aromatik hidrokarbonlar (PAH'lar), SPE numune hazırlama.

INTRODUCTION

The quality and safety of food are significant global concerns, with data on foodborne clinical incidents reaching alarming levels and posing a serious threat to human health. To ensure the safety of the food supply, it is essential to obtain, process and protect food in environments that do not adversely affect human health or create environmental problems. Nutrient contamination can occur at any stage of the production and transportation chain. Similarly, foods can become contaminated by bacteria and other microorganisms, pesticides, food packaging materials, detergents, disinfectants and other cleaning agents. Among the chemical hazards that threaten human health are polycyclic aromatic hydrocarbons (PAHs) (Balcıoğlu and Ceylan, 2019).

Hazelnuts are among the most widely cultivated hard-shelled fruits in the world. Turkey, one of the few countries in the world with favorable weather conditions for

hazelnut production, is at the forefront of world hazelnut production (FAO, 2017). Approximately 2/3 of hazelnut production and exports, which are among the traditional export products, are provided by Turkey (Yıldız, 2020), hazelnut oil is also produced in Turkey. The fat content of hazelnuts, which are rich in macronutrients and micronutrients, varies between 50% and 73% depending on the region, soil and hazelnut variety (Kesen et al., 2016). Hazelnut, which is a nut with a high fat content (~60%), is very rich in oleic, linoleic and palmitic fatty acids. Considering the content of hazelnuts and their effects on human health, they play a key role in human nutrition (Topçu, 2022). Hazelnuts contain high amounts of single and multiple fatty acids known to be effective in preventing heart disease (Crews et al., 2005) and are also good sources of high levels of minerals and B vitamins (B1, B6), vitamin E and natural antioxidants (Alasalvar et al., 2003; Köksal et al., 2006). Hazelnut is a good source of protein containing essential amino acids, mainly arginine, leucine, phenylalanine and valine, in addition to its high-quality oil content. The Hazelnut protein ratio is reported to vary between 9.3% and 22.5% g/100 g (Amaral et al., 2006a; Çetin et al., 2020; Köksal et al., 2006; Şahin et al., 2022; Venkatachalam and Sathe, 2006). They are also characterized by a high fat content with triacylglycerols as the main component and a high proportion of monounsaturated fatty acids, giving hazelnuts high nutritional value. The production of hazelnut crude oil involves the use of physical processes and extraction techniques, which do not involve chemical processing. The high oleic acid content facilitates digestion within the body. The amino acids present in hazelnuts have been demonstrated to exert anticancer, antidiabetic and arteriosclerotic effects (Alasalvar et al., 2003). Hazelnut oil is distinguished by its high content of oleic acid, a monounsaturated fatty acid (Alasalvar et al., 2006; Amaral et al., 2006a; Çetin et al., 2020; Crews et al., 2005). Hazelnut oil, which contains high levels of unsaturated fatty acids and small amounts of saturated fatty acids, has also been demonstrated to exert beneficial effects on human health, including a reduction in the risk of developing cardiovascular disease (Alasalvar and Shahidi, 2009; Mağden, 2023; WHO, 2019).

Polycyclic aromatic hydrocarbons (PAHs) are recognized as hazardous organic pollutants with the potential to cause cancer, genetic mutations and teratogenesis (Sakshi and Haritash, 2020). PAHs are organic compounds comprising at least two benzene rings arranged in angular, linear or cluster chains (Akinpelu et al., 2019; Bianco et al., 2023). Soil quality is a critical factor for plant, animal and human life, and soil pollution has detrimental consequences for a wide range of living organisms, with serious implications for human health as humans are the last link in the food chain. The levels of PAHs present within soil are influenced by various factors, including proximity to the source of pollution, temperature, and prevailing climatic conditions (Ortiz et al., 2012; Bozlaker et al., 2008; Chen et al., 2011; and Tasdemir and Esen, 2007a). In industrial areas, anthropogenic burning activities and atmospheric deposition constitute an important source of PAH pollution in soils (Smith and Harrison 1996; Lee et al., 2001). In urban areas, the use of fossil fuels for heating purposes and traffic density cause the PAH concentrations in the soils of the region to increase day by day (Nadal et al., 2004; Bozlaker et al., 2008).

PAHs are complex mixtures of light and heavy compounds in various categories of foods and beverages (Purcaro et al., 2013). PAHs are derived from the incomplete combustion of organic matter during industrial processes and other human activities (Bansal and Kim, 2015). Food processing, such as smoking, grilling, roasting and frying, can lead to the formation of high amounts of PAHs (Kim et al., 2021; Zelinkova and Wenzl, 2015). Unfortunately, edible vegetable oils, in addition to many other nutrients, are highly susceptible to organic contaminants due to their hydrophobic nature, and PAHs with high lipophilicity are considered a type of food contaminant commonly found in edible oils (Galeotti et al., 2017; Jing et al., 2021). PAH compounds have the potential to contaminate crops through air, water and soil in areas with industrial production. Many vegetable oils are contaminated by the environment during seed growth and coexist with hazardous substances such as mycotoxins, heavy metals, PAHs and pesticide residues (Ji et al., 2023). PAHs enter the human food chain extremely easily through the consumption of vegetable oils, posing a serious threat to human health. In recent years, serious concerns have been raised by consumers and health professionals about the presence of various toxigenic PAHs in foods.

In recent years, there has been considerable interest in PAH studies in Turkey, as well as worldwide. A substantial body of research has demonstrated that edible oils are susceptible to contamination by polycyclic aromatic hydrocarbons (PAHs). This finding has been corroborated by studies conducted by Camargo et al., (2012), Tfouni et al., (2014), Molle et al., (2017) and Ji et al., (2022). Furthermore, numerous studies have been conducted to characterize the lipid fraction of hazelnuts and evaluate its stability during shelf storage (Cialiè Rosso et al., 2021; Ghirardello et al., 2014; Savage et al., 1997; Turan, 2018). This study focuses on hazelnut plants and hazelnut oil, which are among the most important plants in our country, with production and export shares of over 70% worldwide. For this purpose, hazelnut samples were obtained from a garden in the Piraziz district of Giresun Province, It was aimed to monitor the PAH changes that may occur in the oil of

hazelnuts, which are expected to depend on the storage time and condition, and analyzed by GC-MS in this line. In addition to the analysis of the fatty acid methyl ester content of the hazelnut oil (FAME analysis), an attempt was made to determine the PAH content and accumulation.

MATERIAL AND METHOD

Chemicals and standards: The solvents hexane, dichloromethane, and acetone, which were grade for liquid chromatography, and all other chemicals used were purchased from Merck (Darmstadt, Germany). A PAH standard mixture that contained 16 compounds (100 ng/mL; 96–99.9% purity) was obtained from Sigma-Aldrich (Missouri, USA). (St. Louis, MO, USA). These compounds included naphthalene (Nap), acenaphthene (Ace), fluorene (Fle), phenanthrene (Phe), anthracene (Ant), fluoranthene (Flu), pyrene (Pyr), benz(a)anthracene (BaA), chrysene (Chr), benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF), benzo(a)pyrene (BaP), dibenz(a,h)anthracene (DahA), indeno(1,2,3-cd)pyrene (IcdP) and benzo(g,h,i)perylene (BghiP). Deionized water (resistivity of 18.2 MU cm-1) was produced by a Milli-Q water purification system (Millipore Co., USA).

Standard Preparation and Calibration Curves: The standard PAH solution (100 ng/mL) was diluted and stored in the dark at -20 °C. The six-point calibration curves corresponding to the PAH compounds were prepared at different concentrations because the concentration of each PAH analyte in the standard mixture is different. Each point was the average of three injections, and the target analytes were quantified via the external calibration method.

Sample collection and preparation for PAHs: Hazelnut samples were collected from the same nut trees in the Piraziz district of Giresun, Eastern Black Sea Region, during the harvest periods of 2022 (S1) and 2023 (S2) (Fig. 1) and extracted with hexane for 8 hours by Soxhlet to obtain oil. S1 hazelnuts were kept in a sack in the warehouse for a year, as the people did. A 5 g oil sample was weighed into a 10 mL volumetric flask, diluted to volume with n-hekzan and sonicated in an ultrasonic bath. Then, a 1.0 mL sample was extracted in an SPE cartridge previously washed with 20 mL of dichloromethane, dried completely by means of vacuum, and conditioned with 20 mL of n-hexane. The mixture of n-hexane: dichloramethane was 70:30 (v/v). PAHs were eluted, and the first 8 mL of eluent was discharged. The following 8 mL fraction was collected and concentrated under N_2 to approximately 20-30 µL, and the last residue was dissolved in 100 µL ACN and run in a GC-MS analysis system (Ergönül and Sanchez, 2013; Teixeira et al., 2007).

Apparatus and GC-MS System: The samples were filtered with SPE cartridges packed with a silica phase (Chromabond C18, octadecyl-modified silica, Germany) PAH mixture, and the samples were analyzed by a GC–MS instrument system (Agilent-Model 7890A-5975C inert MSD) in selected ion monitoring (SIM) acquisition mode. The method conditions of the chromatographic system were as follows: carrier gas, helium; flow rate, 1.5 mL/min; and initial injection port temperature, 30 °C. One microliter of the sample was run in pulsed splitless mode. An Agilent 5% HP-5MS phenyl methyl Silox column (30 m × 250 µm × 0.25 µm) was used, and the pressure was 18.635 psi. The GC oven parameters were set to increase from 55 °C for 1 min, then 25 °C/min to 320 °C for 5 min, and finally increased to 320 °C at 16.6 min. The mean recovery of PAHs ranged from 85.23% to 101.36% in this study (Table 1). Each standard mixture at different concentrations was analyzed in triplicate, and the standard deviation was < 5%.

Fig 1. Locations of the hazelnut samples.
Şekil 1. Fındık örneklerinin yerleşim yerleri.

RESULTS AND DISCUSSION

PAH analysis: Table 1 shows the results for the RT, LOD and LOQ ranges. The detection limits ranged from 0.25 ng/g to 0.43 ng/g. The mean recovery of PAHs ranged from 85.23% to 102.64% in this study (Table 2).

Table 1. Methodical description of PAH standard.

PAH	RT (min)	Identification (ion)	LOD (ng/g)	LOQ (ng/g)
Nap	5.544	(128) 129.127.102	0.21	0.50
Acy	6.306	(152) 151.150.153	0.22	0.55
Ace	6.486	(153) 154.152.151	0.25	0.60
Flu	6.977	(166) 165.167.163	0.35	0.70
Phe	7.898	(178) 176.179.152	0.36	0.72
Ant	7.941	(178) 176.179.152	0.31	0.65
Flua	9.049	(202) 203.200.101	0.24	0.58
Pyr	9.266	(202) 203.200.101	0.33	0.68
BaA	10.427	(228) 226.229.113	0.35	0.70
Chr	10.468	(228) 226.229.113	0.41	0.80
BpF	11.408	(252) 253.250.126	0.43	0.82
BkF	11.430	(252) 253.250.126	0.40	0.78
BaP	11.683	(252) 253.250.126	0.25	0.60
Inp	12.730	(276) 138.274.277	0.32	0.68
BghiP	12.997	(278) 276.279.139	0.34	0.69
DahA	13.021	(276) 138.274.277	0.30	0.62

Generally people who own hazelnut gardens prefer to keep hazelnuts for 1 year and sell them to be more economically profitable. Hazelnut samples collected in 2022 and 2023. PAH changes were investigated and analyzed. The

effects of 1 year of storage on the PAH contents and the total PAH contents are given in Table 3.

Table 2. Certified and observed concentrations (µg/mL) and % recoveries of all PAH analytes.

PAH	Certified	Observed	% Recovery
Nap	998.6	992.4	99.38
Acy	2000.2	1830.2	91.50
Ace	995.4	1002.5	100.71
Flu	198.6	201.3	101.36
Phe	98.7	95.6	96.86
Ant	99	98.1	99.09
Flua	196.2	197.5	100.66
Pyr	98.5	101.1	102.64
BaA	100.3	100.6	100.30
Chr	99.7	100.2	100.50
BpF	199.4	196.5	98.55
BkF	99.9	99.4	99.50
BaP	99.6	99.4	99.80
Inp	99.9	85.14	85.23
DahA	199.8	200.6	100.40
BghiP	199.2	201.4	101.10

Table 3. PAH16 components of the samples (ng/kg).

ng/kg	S-1	S-2
Naphthalene	7.834±0.015	5.335±0.021
Acenaphthylene	0.152±0.010	0.144±0.013
Acenaphthene	0.015±0.001	0.036±0.012
Fluorene	0.022±0.012	0.018±0.015
Phenanthrene	0.057±0.000	0.042±0.016
Anthracene	ND	ND
Fluoranthene	0.024±0.001	0.027±0.014
Pyrene	0.024±0.001	0.025±0.045
Benzo(a)anthracene	0.008±0.001	0.052±0.051
Chrysene	0.008±0.001	0.062±0.014
Benzo(b)fluoranthene	0.116±0.014	0.120±0.027
Benzo(k)fluoranthene	0.059±0.001	0.061±0.014
Benzo(a)pyrene	ND	ND
Dibenzo(a.h)anthracene	0.076±0.001	0.104±0.029
Benzo(g.h.i)perylene	0.088±0.001	0.047±0.010
Indenol(1.2.3-CD)pyrene	ND	ND
Σ16PAHs	8.483±0.032	6.075±0.024

The samples were harvested during the 2022 (S1) and 2023 (S2) seasons.

The detection rate of naphthalene was the highest among the PAH concentrations (S-1: 7,834 and S-2:5,335). Naphthalene was the most abundant PAH analyte, with 92.35% in sample S1 and 87.82% in sample S2. The sensitivity of hydrocarbons to microbial adsorption varies according to differences in their structure. These sensitivities can be listed in the following order: n-alkanes > branched alkanes > small-molecular-weight aromatics > cycloalkanes (Perry, 1984). The highest degradation rates were observed for the soluble compounds. This is followed by light aromatic compounds and high-molecular-weight aromatic compounds (Jobson et al., 1972). Occurrence rates of high-molecular-weight polycyclic aromatic hydrocarbons including naphthalene and phenanthrene were found to be associated with their dissolution in water rather than the total substance concentration (Thomas et al., 1986). The high level of naphthene observed in our study can be attributed to the factors like these (Jobson et al., 1972; Perry, 1984; Thomas et al., 1986). The Σ16PAH concentration in S1 was higher than that in S2 (Table 3). This may be due to the fact that all samples were selected from the same orchard; a one-year holding period would include samples collected from different areas and with different storage conditions, giving results that can be assessed statistically. In a study fifteen vegetable oils and butters were analyzed to determine the PAH4 (benz[a]anthracene, chrysene, benzo[b]fluoranthene and benzo[a]pyrene) contents of butters and vegetable oils (olive oils, palm oil, sunflower oil, almond oil, hazelnut oil and coffee oil) offered for sale in the Turkish market. The highest amount of PAH4 (20.76 µg kg^{-1}) was detected in sunflower oil, followed by olive oil (9.51 µg kg^{-1}), palm oil (6.05 µg kg^{-1}), coffee oil (5.25 µg kg^{-1}) and hazelnut oil (1.95 µg kg^{-1}). In another study, HPLC analysis was performed for the detection of benzo(a)pyrene (BaP) in 40 oil samples, and the concentration of BaP was calculated as 4.80 ± 0.01 ppb for hazelnut oil (Gülten et al., 2007). The SCF recommended the use of benzo(a)pyrene (BaP) as a marker for the presence and effects of carcinogenic PAHs in food, based on the evaluation of PAH profiles in food (European Food Safety Authority, 2008). According to the law, the maximum BaP level is 2 µg kg^{-1}, but BaP was not detected in either oil sample.

Fatty acid composition: Fatty acid composition was determined according to the methods of Oliveira et al. (2008), and Thermo Scientific Application Notes with some modifications. A sample of 100 µL of oil was used for fatty acid methyl esterification with 1 mL of methyl esterification reagent (2 N KOH solution in CH_3OH). The fatty acid profile was analyzed by an Agilent GC-7890 instrument with a chromatographic column (Thermo, 10 m × 0.10 mm ID × 0.20 µm film, Agilent, USA). The results are expressed as a percentage of the fatty acid composition.

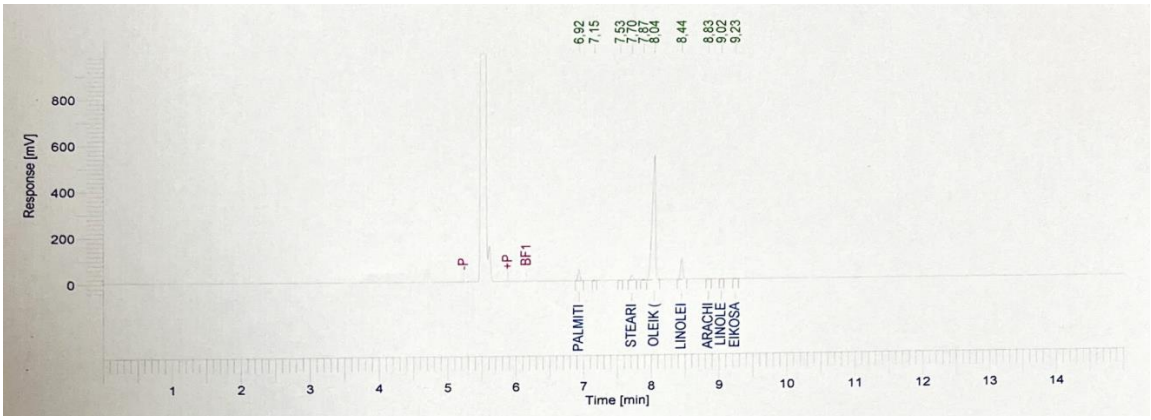

Fig. 3. Fatty acid composition of sample S2 (2023 harvest)

Table 4. Changes in fatty acid composition (%).
Tablo 4. Yağ asidi bileşimindeki değişimler (%).

% Fatty acids	S1	S2
Palmitic acid (C16:0)	5.95±0.01	4.61±0.02
Stearic acid (C18:0)	2.55±0.02	2.24±0.05
Oleic acid (C18:1)	84.2±0.12	82.81±0.13
Linoleic acid (C18:2)	7.3±0.02	10.05±0.06
Arachidik acid (C20:0)	-	0.09±0.01
Linolenic acid (C18:3. n-6)	-	0.07±0.01
Eicosanoic acid (C20:1)	-	0.13±0.01

The values are expressed as the means ± SDs (n = 3). (S1: 2022 harvest. S2: 2023 harvest).

Other researchers have reported similar results with respect to the fatty acid composition of other hazelnut oils (Amaral et al., 2006; Turan, 2018). The concentration of palmitic acid was found to be significantly greater in the oil of hazelnuts that had been stored for one year. A similar trend was observed for oleic acid, stearic acid and arachidic acid. Linolenic acid and eicosanoic acid were not detected in the oil of hazelnuts that had been stored for one year. Fresh hazelnut oil was used. The concentrations of these three fatty acids did not exceed 0.2% (Table 4). The extent of the degradation of linoleic and linolenic acids to monounsaturated acids was greater than that of oleic acid during the specified period. This result is consistent with those previously reported by Guillén et al., (2009) in which linoleic acid was oxidized throughout the oxidation process of sunflower oil, resulting in an increased oleic acid content.

CONCLUSION

A review of the literature revealed a limited scope of PAH analyses in hazelnut oil. In particular the 16-component PAH analysis was not identified as a topic of investigation within the literature. The objective of this study was to analyze the nature of the change in the PAH content of hazelnut oil as a result of storage with the aim of providing a reference point for hazelnut owners. Benzo[a]pyrene, a known carcinogen, was not detected in either sample, however naphthalene formation increased the amount of Σ16PAH. Furthermore, the variation in fatty acid composition was also examined. This condition was determined to be a consequence of the hazelnut being stored in a sack for one year in an environment with uncontrolled humidity. It is thought that storing hazelnut samples in temperature and moisture controlled stores will prevent the formation of naphthalene.

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