Further Evidence on the Removal of Polycyclic Aromatic Hydrocarbons (PAHs) During Refining of Olive Pomace Oil

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Influences of chemical refining parameters on the removal of 15 polycyclic aromatic hydrocarbons (PAHs) from a list of 16 EPA-priority pollutant PAHs are investigated. For this purpose, various process conditions for each refining stages (degumming, neutralization, bleaching, and deodorization) are applied to crude olive pomace oil and the changes in PAH concentrations are monitored using a high performance liquid chromatography with florescence detector. Results show that total PAH content of the oil is reduced effectively through all refining stages. Degumming has considerable effects on removal of individual PAHs depending upon water or acid application while the most significant reduction in total PAH is achieved when 1% water is used. Neutralization has a significant impact on removal of PAHs from the oil, however, washing steps have limited effects. Using various amounts (0.3, 0.6, and 0.9%) of activated carbon in 3% of bleaching earth are more effective on the reduction of total and heavy PAHs from the oil and the most reduction is achieved when 0.9% activated carbon is used. Deodorization is effective mainly on decreases in light PAHs while temperature incrementation does not have any significant impact on the reduction in total PAH content. *Practical Applications*: Olive pomace oil may include high amounts of PAHs. Because of their potential carcinogenic character, removal of PAHs from the oils has a great importance for oil acceptability. Results of this study provide information about the effects of process conditions of degumming, neutralization, bleaching, and deodorization on PAH reduction in olive pomace oil. These results might open up new process strategies in production of high quality olive pomace oil via refining.

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1. Introduction

Polycyclic aromatic hydrocarbons (PAHs), composed of two or more fused aromatic rings with different functional groups were reported as carcinogenic and mutagenic.[1] Edible oils are easily contaminated with PAHs because of their lipophilic nature.[2] More than 100 PAHs have been found in environment as a result of incomplete combustion of organic matter.[3] Due to their carcinogenic activity, 16 of these have been selected as priority pollutants by United States Environmental Protection Agency (US EPA).[4]

Foods may be contaminated by these substances not only from environment but also from various industrial process and cooking conditions.[5–7] It is well known that PAHs might be found in some edible oils and fats at high levels.[8,9] Due to their apolar nature, lipids are the primary source of human exposure to PAHs.[10] Contamination of vegetable oils by PAHs can occur mainly by environmental pollution of the oil bearing plants harvested from contaminated soil, water, and air.[11] Solvents used for oil extraction[12] and drying process of oil seeds at high temperatures[13,14] might be other factors for their contamination. Crude olive pomace oil might have high amount of PAHs, depending on drying conditions of olive pomace before solvent extraction.[15,16]

The International Agency for Research on Cancer (IARC) has established benzo(*a*)pyrene as well-known carcinogen in food products.[17] The European Union (Commission Regulation EC No. 208/2005) has listed maximum level of benzo(*a*)pyrene as $2 \, \mu g \, kg^{-1}$ in oils and fats for direct consumption or use as an ingredient in foods.[18] Commission Regulation (EU) 835/2011 indicated that instead of benzo[*a*]pyrene as a suitable marker for the occurrence and toxicity of PAHs in food, four specific PAHs (benzo[*a*]pyrene, benzo[*a*]anthracene, benzo[*b*]fluoranthene, and chrysene) would be the most suitable indicators of PAHs in food with the maximum levels for the sum of them as $10 \, mg \, kg^{-1}$.[19]

Due to the vegetable oil acceptability by consumers, there is an urgent need to reduce toxic and harmful contaminants in vegetable oils. The refining process can reduce most of the

contaminants and improve the oil quality.[19] Refining consist of a series of operations including degumming, neutralization, bleaching, and deodorization to remove the undesirable impurities.[20] Some researchers have reported the impacts of refining on removal of PAHs and the majority of them determined the effects of refining steps on the PAH contents of the samples collected from refineries. Virgin and refined olive oils, crude and refined olive pomace oils collected from local producers were subjected to analyze for their PAHs contents.[8,16] Effects of refining stages on removal of PAHs from vegetable oils obtained from different steps of commercial refining were revealed by some researchers that neutralization and deodorization steps provided strong reductions in PAH content of the oils.[9,21–23] Efficiencies of some bleaching earths for elimination of benzo-(α)-pyrene from olive pomace oil were also reported.[24] To our best knowledge, no study had focused on the influence of refining parameters on the reduction of PAHs which are on the priority pollutant list of US EPA. In this study, it was aimed to investigate the impacts of chemical refining parameters on the removal of PAHs from vegetable oil. For this aim, crude olive pomace oil was subjected to various parameters during chemical refining stages such as degumming, neutralization, bleaching, and deodorization.

2. Experimental Section

2.1. Chemicals

Sodium hydroxide and HPLC grade water were purchased from Merck (Germany); acetonitrile, dichloromethane, n-hexane, chloroform, acetic acid, ethanol, acetone were obtained from Sigma–Aldrich (St. Louis, MO, USA). All chemicals used for the analyses of PAHs were HPLC grade and all other chemicals were analytical grade. The 15 polycyclic aromatic hydrocarbons from the list of 16 EPA-priority pollutant PAHs (naphthalene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenz(a,h) anthracene, benzo(g,h,i)perylene, and indeno(1,2,3-cd)pyrene) were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). The PAH standards were stored at −18 °C in dark to prevent undesirable effect of light and temperature. Glassware used was washed with detergent and water, followed by rinsing with acetone and dried before use.

2.2. Samples

Crude olive pomace oil including 12.58% of FFA and 22080 μg kg^{-1} of total PAHs was obtained from Verde Oil Company (İzmir, Turkey). Bleaching earth (F16) and activated carbon (PAK C 1200 S PAH) were supplied by Damla Kimya (Ankara, Turkey).

2.3. Lab Scale Refining

Crude olive pomace oil was degummed (water and acid degumming), neutralized, bleached, and deodorized using lab scale refining units according to the modified methods of Zacchi and Eggers.[25]

i) *Degumming:* Crude olive pomace oil was degummed with different concentrations of phosphoric acid and water. The oil was heated up to 80 °C and 85% phosphoric acid (0.1, 0.2, and 0.3% v/w) or distilled water (1 and 2% v/w) were introduced into oil under gentle agitation. The mixture was then stirred at 80 °C for 30 min and cooled to room temperature before centrifugation (30 min, 3600 rpm) to separate the gums from the oil.

ii) *Neutralization:* Crude olive pomace oil was heated up to 70 °C and required amount of sodium hydroxide lye (24 Bé) plus 10% in excess was added into oil. The AOCS official method Ca 9c-52 was used for the calculations of the required amounts of caustic solution to neutralize the free fatty acids in 100 g crude oil.[26] Then constant reaction time as 30 min was applied under agitation. The soap was then removed from the neutralized oil by centrifugation and the oil was washed three times with 30% of water (v/w) in order to remove residual soap.

iii) *Bleaching:* Neutralized oil was bleached using 3% bleaching earth with elevated activated carbon content (0.3, 0.6, and 0.9%) under vacuum. Oil was heated up to 105 °C and bleaching earth or bleaching earth-activated carbon mixture was introduced into oil. A slurry was stirred for 15 min at 105–110 °C. After the treatment time, bleaching earth and activated carbon were filtered through Whatman No: 1 filter paper.

iv) *Deodorization:* The deodorization was carried out using a 1 L capacity laboratory-scale deodorizer. Bleached oil was placed in the flask and heated up to the 220, 230, and 240 °C under a 3 mbar pressure for 120 min. Steam produced from distilled water was introduced into oil (1%, v/w) during deodorization.

2.4. Analysis of PAHs Content

2.4.1. Extraction and Purification

For extraction and clean-up procedure, a solid phase extraction (SPE) method previously described by Moret and Conte[27] with a minor modification (amount of oil sample) was used. Purification was performed using a SPE mini column in a visiprep solid-phase extraction Vacuum Manifold (Supelco, USA). Oil sample (0.25 g) was diluted with n-hexane in 10 mL volumetric flask. Then, 1.0 mL of the sample solution was transferred onto a 5-g silica SPE cartridge (Mega Bond Elut, 20 mL, Varian, Palo Alto, CA, USA) previously conditioned with 20 mL of dichloromethane, dried completely under vacuum and conditioned with 20 mL of n-hexane. PAHs solution was eluted with mixture of n-hexane and dichloromethane (70:30 v/v). The first 8 mL of elute was discarded, and the following 8 mL elution including PAH fraction was collected in a tube. The flow rate was adjusted to one drop per second. Elute was evaporated with nitrogen. The concentrate was dissolved in 1 mL of acetonitrile and injected into the HPLC.

2.4.2. HPLC-FLD Analysis

The analytical determination of PAHs was carried out using an HPLC (Shimadzu, Kyoto, Japan) equipped with a fluorescence detector (Model RF-20AXS Shimadzu, Kyoto, Japan). A Pinnacle II PAH column (4 mm, 50 mm, 2.1 mm, Restek) at 18 °C was used along with a security guard column (Trident Direct Filter 4 mm, 2 mm) for separation. Injection volume was 20 µL. The mobile phase of gradient program was composed of acetonitrile (A) and water (B). The solvent system was started with 60% acetonitrile (A) and 40% water (B) and continued with a gradient program to obtain 60% (A) at 3 min, 90% (A) at 14 min, 100% (A) at 26 min, 60% (A) at 28 min until the end of the run with the flow rate of 0.2 mL min^{-1}. Total time of analyses was 34 min. Excitation (260 nm) and emission (375 and 460 nm) wavelengths channels were used. Acanaphthylene (An) did not give fluorescence at working wavelengths so it could not be detected in the system.[8,9] Therefore, only 15 PAHs reported above were identified. PAHs were quantified based on the peak areas compared with those of external standards.

2.5. Statistical Analysis

All data shown represent the mean values ± standard deviation of duplicate measurements. PAHs content of the samples were analyzed by analysis of variance (ANOVA) using SPSS version 20 (SPSS Inc., Chicago, IL, USA). The differences between mean values were compared using Duncan's multiple-range test with a level of significance of $p < 0.05$.

3. Results and Discussion

Degumming is a crucial step in the refining process where gums such as phosphatides are removed from crude oil. Water and/or acid degumming are applied for soft oils depending on phosphatide type and content. In this study, water (1, 2%) and phosphoric acid (0.1, 0.2, and 0.3%) were used for the degumming of crude olive pomace oil that may contain high amount of total 15 PAHs (Np, Anp, Flr, Phe, Ant, Flu, Pyr, BaA, Chr, BbF, BkF, BaP, DahA, BghiP, and IcdP) as reported by Kiralan et al.,[13] most of which are possibly formed during air drying at high temperatures applied before solvent extraction. Crude olive pomace oil has to be refined before human consumption and refining process should also reduce PAHs content less than maximum allowable limit of 10 ppb for total of four PAHs, as indicated in EU Regulation.[19]

All degumming processes showed significant reduction of all PAH levels ($p < 0.05$) (**Figure 1**A and B). Flr, Phe, and Chr were the main PAHs because their concentration was more than 2000 µg kg^{-1} in crude olive pomace oil. As observed in Figure 1A, water degumming was more effective to reduce the amounts of Flr and Phe than the phosphoric acid degumming. Even though all degumming process showed significant reduction of Chr level, phosphoric acid applications led to more Chr reduction than water degumming (Figure 1B) when used more than 0.2%. **Figure 2** shows the reductions in total PAHs content in crude olive pomace oil with degumming process. As shown in Figure 2, total PAH reductions were more than 50% in all degumming processes (water and acid degumming) and calculated as 64, 79, 62, 82, and 79% for 0.1, 0.2, and 0.3% of phosphoric acid and 1 and 2% of water
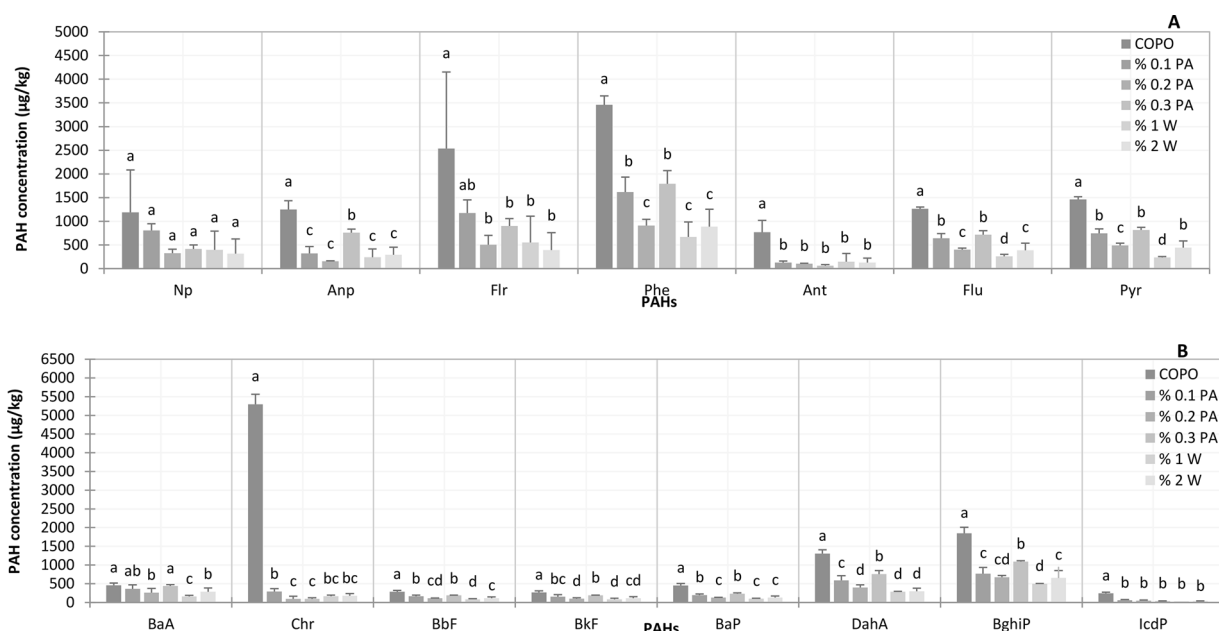

Figure 1. Changes in light (A) and heavy PAHs (B) concentrations in crude olive pomace oil after acid and water degumming. Error bars represent standard deviation obtained from duplicate ($n = 2$) experiments. Bars with different letters show significant differences ($p < 0.05$). (COPO, crude olive pomace oil; PA, phosphoric acid; W, water).
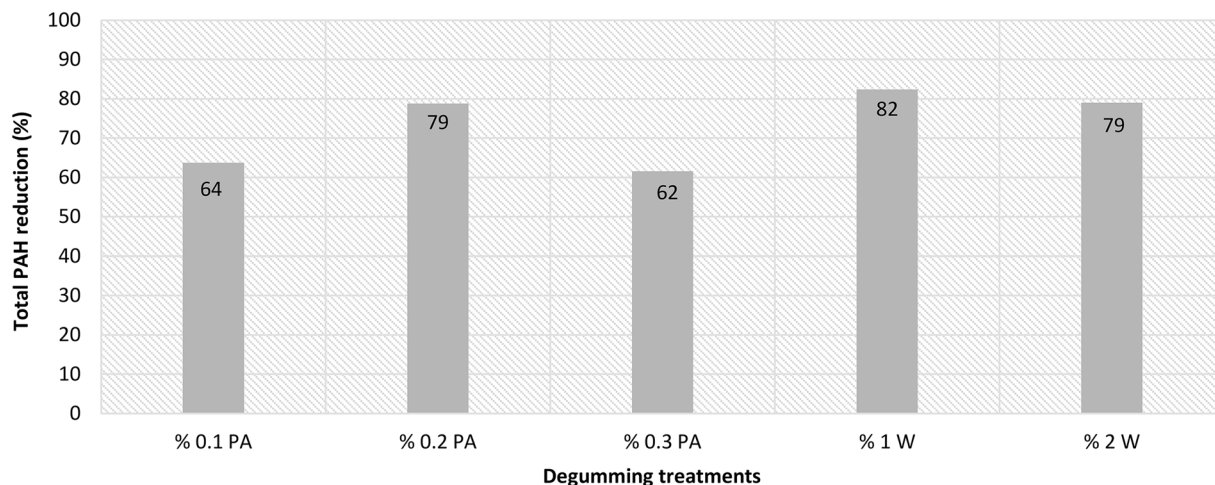
Figure 2. Removals of total PAHs in crude olive pomace oils after acid and water degumming. PA, phosphoric acid; W, water.

treatments, respectively. The most significant reduction in total PAH was achieved 82% when 1% water was used in degumming process.

Degumming step is generally skipped during chemical refining of olive pomace oils. Therefore, neutralization step was performed using crude olive pomace oil as well. In this process, 12.58% of free fatty acids in the oil was neutralized with a calculated caustic solution. After centrifugation, the mixture was washed three times to remove soap residue. **Figure 3** shows the effect of this stage on the reduction of PAH content in crude olive pomace oil. As shown in Figure 3, the concentrations of all

light (Np, Anp, Flr, Phe, Ant, Flu, Pyr) (Figure 3A) and heavy (BaA, Chr, BbF, BkF, BaP, DahA, BghiP) (Figure 3B) PAHs were decreased significantly with the neutralization ($p < 0.05$). Results also show that neutralization contributed to sharp decrease in most individual light and heavy PAHs as well. However, there were almost no significant decreases in the PAH contents after washing steps ($p > 0.05$). As observed in **Figure 4**, neutralization process played an important role in removing total PAH content from the oil. After neutralization process, total PAH content was reduced more than 90%, which suggests that neutralization is a significant step for mitigating PAHs in the oil. Previous studies
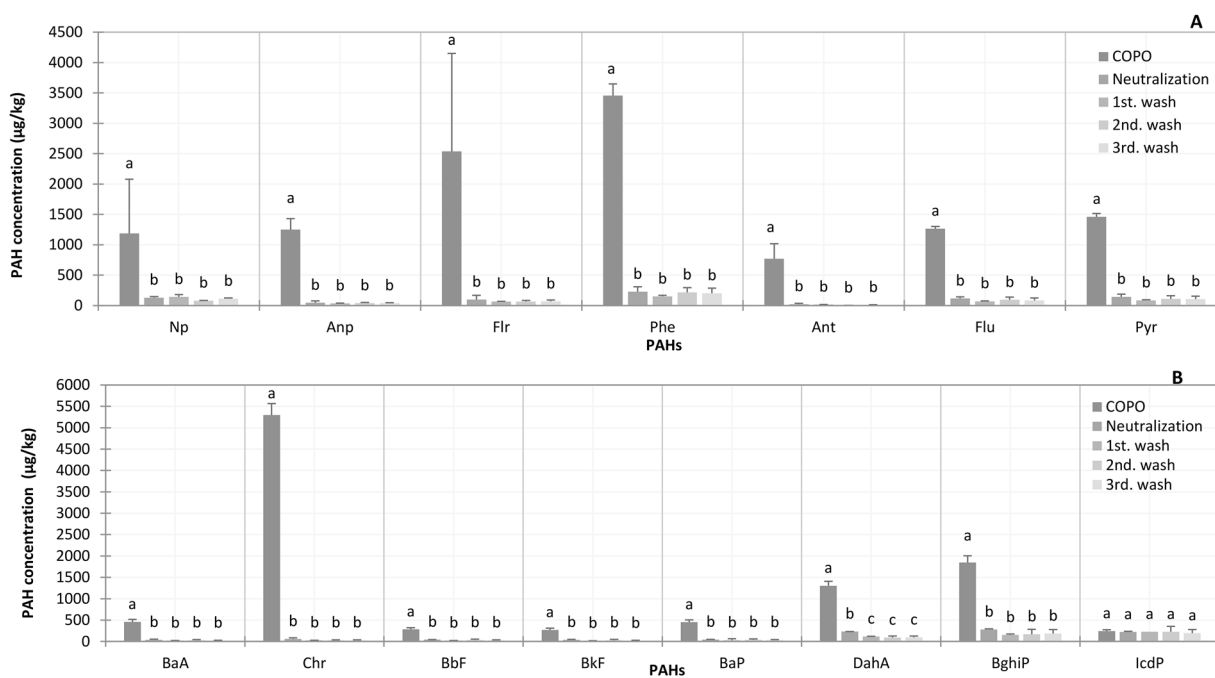

Figure 3. Changes in light (A) and heavy PAHs (B) concentrations in olive pomace oil after neutralization. Error bars represent standard deviation obtained from duplicate ($n = 2$) experiments. Bars with different letters show significant differences ($p < 0.05$). (COPO, crude olive pomace oil).
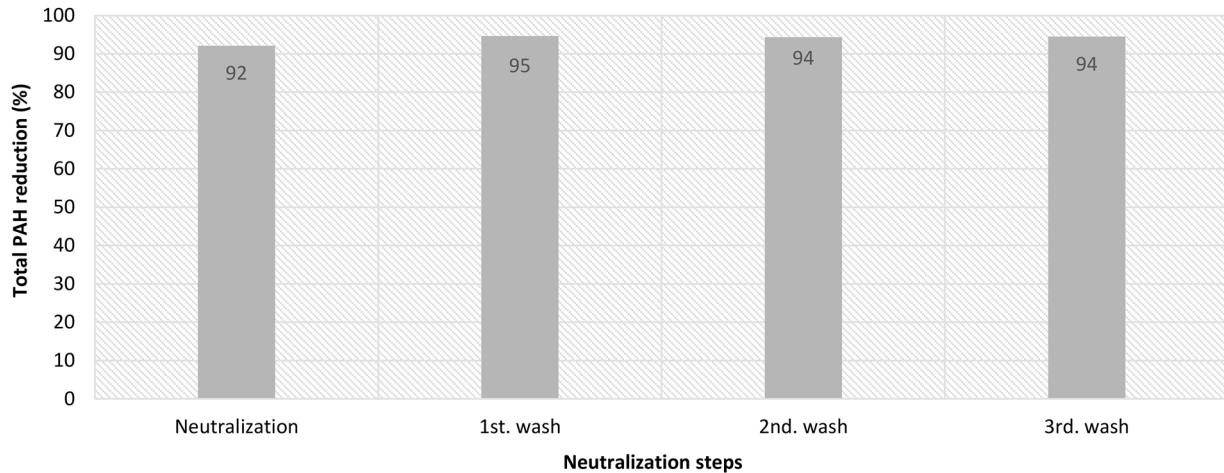
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Figure 4. Removals of total PAHs in olive pomace oils after neutralization.

showed that refining reduced PAHs significantly in the oil and a considerable reduction was achieved by neutralization.[9,21,23]

The influences of bleaching earth and activated carbon on reduction of PAH content in neutralized olive pomace oil are shown in **Figure 5**. Same amount of bleaching earth (3%) was used in all bleaching applications with various activated carbon ratio (0.3, 0.6, 0.9%). It was observed that bleaching earth and activated carbon applications were significantly effective to reduce light and heavy PAHs from the oil ($p < 0.05$). As shown in Figure 5A, Phe, Flu, and Pyr were dominant light PAHs in

neutralized olive pomace oil, which were more than $300 \, \mu g \, kg^{-1}$ in the oil. After bleaching with bleaching earth, their concentrations were reduced significantly ($p < 0.05$). Results indicated that using activated carbon with bleaching earth was more effective to reduce the amounts of heavy PAHs (Chr, BbF, BkF, BaP, DahA, BghiP, and IcdP) rather than using only bleaching earth. All activated carbon applications (0.3, 0.6, and 0.9%) completely removed DahA, BghiP, and IcdP from the oil. Bleaching with 3% of bleaching earth resulted in a 33% of reduction in total PAH content. However, these reductions were
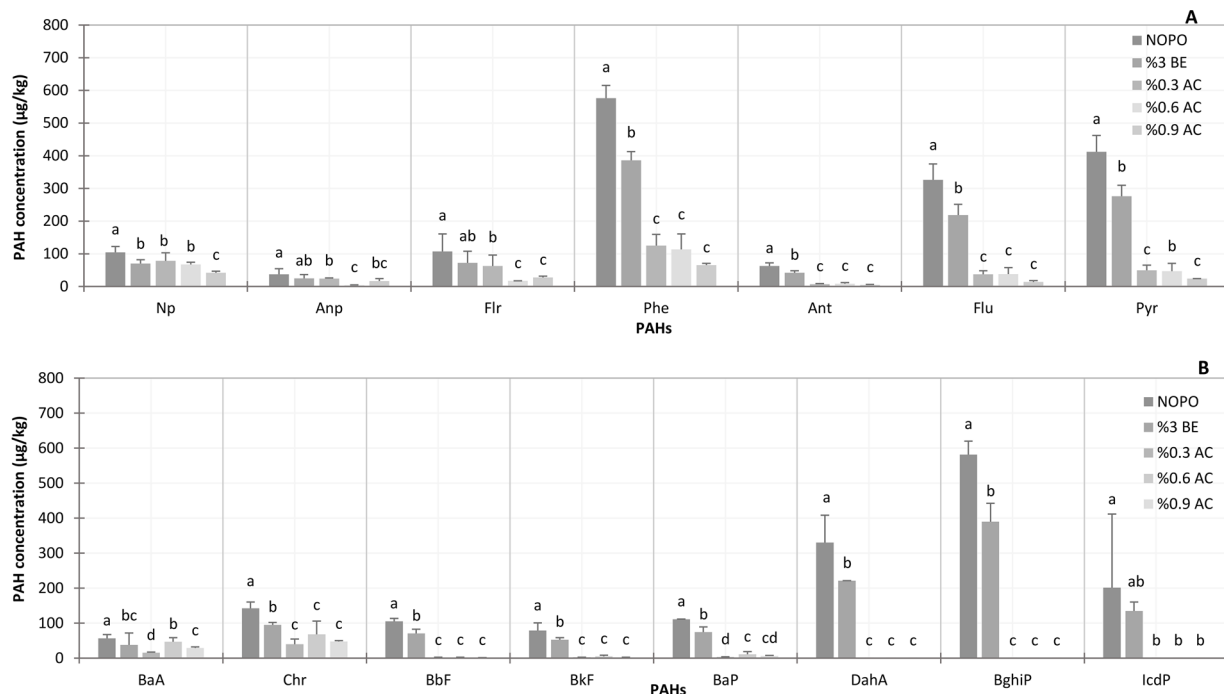

Figure 5. Changes in light (A) and heavy PAHs (B) concentrations in olive pomace oil after bleaching. Error bars represent standard deviation obtained from duplicate ($n = 2$) experiments. Bars with different letters show significant differences ($p < 0.05$). (NOPO, neutralized olive pomace oil; BE, bleaching earth; AC, activated carbon).
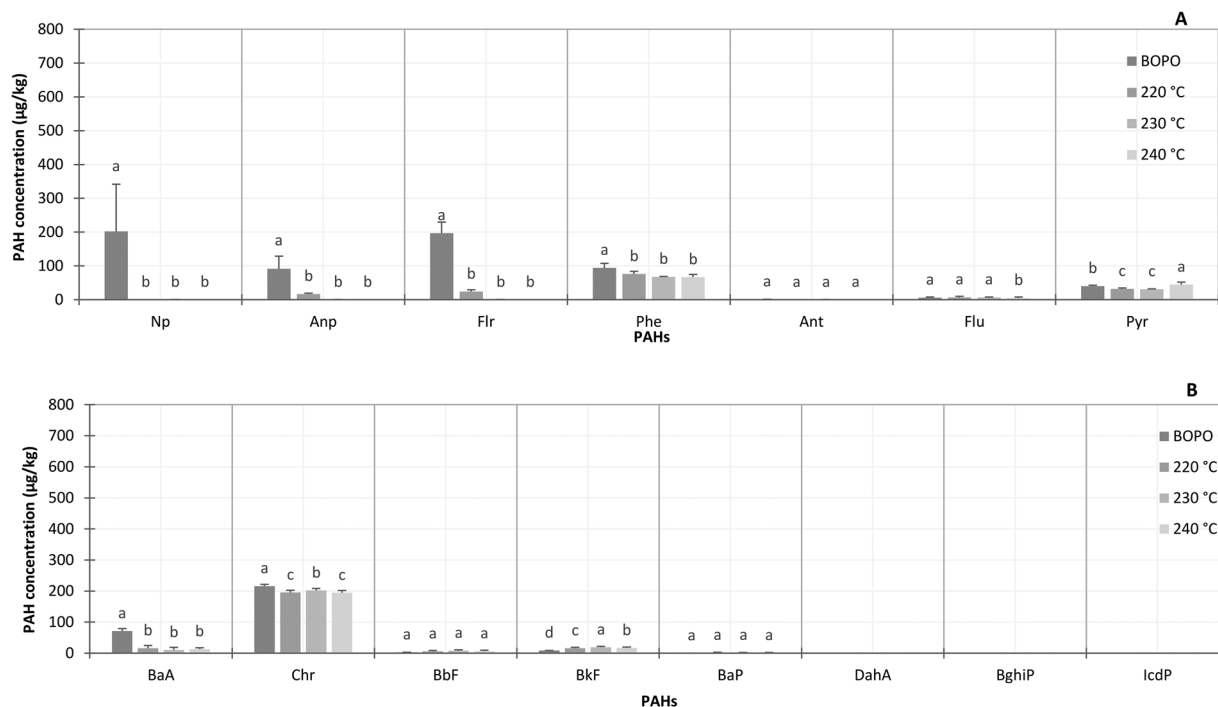
Figure 6. Changes in light (A) and heavy PAHs (B) concentrations in olive pomace oil during deodorization. Error bars represent standard deviation obtained from duplicate ($n = 2$) experiments. Bars with different letters show significant differences ($p < 0.05$). (BOPO, bleached olive pomace oil).

increased to 86, 87, and 91% when 0.3, 0.6, and 0.9% activated carbon were used, respectively. Similar results were obtained by other researchers[28] who found that PAHs adsorbtion on activated carbon was affected by initial PAH concentration of the oil and activated carbon dosage. As reported in some previous studies, contaminants such as PAHs were removed with bleaching stage effectively from vegetable oils.[21,24,29]

Figure 6 shows the effects of different deodorization temperatures on the reduction of PAHs in bleached olive pomace oil. Bleached oil was deodorized at 220, 230, and 240 °C under 3 mbar for 120 min. As indicated in Figure 6A and B, the concentrations of some light PAHs (Np, Anp, Flr, Phe, and Ant) and BaA were removed effectively at 220 °C. When the deodorization temperature was increased to 230 and 240 °C, the amount of Np, Anp, and Flr were completely removed from the oil. Total PAH reductions after deodorization were recorded as 47, 53, and 53% for deodorization temperatures at 220, 230, and 240 °C, respectively. The results showed that deodorization process removed light PAHs effectively from vegetable oils, which was also reported by other authors.[9,30]

4. Conclusions

This study has shown that refining stages (degumming, neutralization, bleaching, and deodorization) were effective in reducing PAH content in olive pomace oil. Refining parameters have also important effects on reducing PAHs. Degumming process contributes to significant PAH removal from crude olive pomace oil, depending on the dosage of acid or water. Water degumming was found more effective. Strong reduction in total PAH content was observed after neutralization. However, washing with water did not have any significant effect on the removal of PAHs. Using activated carbon with bleaching earth and increasing activated carbon amount enhance the effectiveness of bleaching on reduction of PAHs in olive pomace oil. Deodorization removed mainly light PAHs, however, raising the temperature during this process was not effective on the removal of total PAHs.

Abbreviations

Anp, acenaphthene; Ant, anthracene; BaA, benzo(*a*)anthracene; BaP, benzo[*a*]pyrene; BbF, benzo(*b*)fluoranthene; BghiP, benzo(*g,h,l*)perylene; BkF, benzo(*k*)fluoranthene; Chr, chrysene; DahA, dibenzo(*a,h*)anthracene; EFSA, European Food Safety Authority; Flr, fluorene; Flu, fluoranthrene; EPA, Environmental Protection Agency; HPLC, high performance liquid chromatography; HPLC-FLD, high performance liquid chromatography-fluorescence detection; IARC, International Agency for Research on Cancer; IcdP, indeno(1.2.3-*cd*)pyrene; Np, naphthalene; PAH, polycyclic aromatic hydrocarbons; Phe, phenanthrene; Pyr, pyrene; US EPA, United States Environmental Protection Agency.

Conflict of Interest

The authors declare no conflict of interest.

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