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Investigation of Polycyclic Aromatic Hydrocarbons (PAH) Levels in Trout Caught with Different Wood-Chips

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Abstract: Carcinogenic polycyclic aromatic hydrocarbons (PAHs) that threaten human health are found in smoked products. Mugla has higher production potential regarding both sea bream and sea bass, and trout culture. This study investigated PAHs and other aromatic compounds in natural and cultured abalone fishes smoked with various wood fires. In sensory analyses, trout smoked with olive wood sawdust was the most popular, with a rate of 60.1%, while oak was the second most popular, with a rate of 46.6%. According to the results obtained from chemical analyses, benzo[α]pyrene, known as polycyclic aromatic hydrocarbon, was found to be at a trace level (≤0.005 µg/kg) in the skin parts, while 13-(1-naphthyl)-13H-dibenzo[a,i]fluorene was found in the skin of fish smoked with pinar (*Quercus aucheri*) wood in an amount of 3 µg/kg. The same compound was detected at a trace level in the fleshy part, including the skin. When evaluated in terms of aromatic compounds other than PAHs, nicotinamide ranged between 15.57 - 52.84 µg/kg, syringol ranged between 2.77 - 39.46 µg/kg, 2-phenoxy ethanol ranged between 8.11 - 37.72 µg/kg in fish smoked with olive wood, while it was not detected in fish smoked with pinar wood. Considering the amounts of PAH compounds determined, the results are appropriate according to the Turkish Food Codex, and it is revealed that there is no risk for the consumer in terms of health and a new smoked fish product can be obtained with olive wood sawdust, which is expected to be consumed with high appreciation.

Keywords: Polycyclic aromatic hydrocarbons (PAH); trout; olive wood; pinar wood; sensory analysis; syringol.

1. Introduction

From the moment people started to live on earth, they have benefited from nature and ensured the continuation of their vital activities. In times when there is no technology, and the population is low, nature can repair itself against human destruction, but population growth, the development of technology day by day, and the change in people's consumption expenses make it difficult for natural resources to renew themselves. The rapid increase in industrialization and human population has brought along the problem of environmental pollution. This pollution has adversely affected air, soil, and water, which

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threatens the health of living organisms [1-2]. One of the most dangerous diseases today is cancer. Direct exposure to carcinogenic and toxic substances by breathing contaminated air and consuming contaminated food and water. Industrial and industrial waste, exhaust, flue gases, pesticides, and harmful chemical waste from garbage disrupt the balance of nature as they mix with water, air, food, and soil [3]. Most importantly, they threaten human health. Pollutants such as nitrogen oxides, pesticides, sulphur dioxides, and polycyclic aromatic hydrocarbons (PAHs) are carcinogenic to human health [4]. Polycyclic aromatic hydrocarbons are also found in smoked seafood, and their amounts in the products are also important for health. [5-6]. Food preservation by smoking is one of the oldest known preservation methods, and smoked product technology is a much-preferred method in Europe and some Asian countries [7-8-9]. PAHs are harmful organic pollutants found in petroleum and its derivatives, which are released into the environment because of the incomplete combustion of fossil fuels. Most of the PAHs cause environmental pollution and largely affect the biological balance because they can remain in the environment for a long time. PAHs are multi-ring hydrocarbon compounds that are released into the atmosphere as a result of combustion and are a chemical group containing more than a hundred different species. PAHs are generally colorless, white, light yellow, or green. Some PAHs are found in crude oil and tar, while others are used in the production of pharmaceuticals, paints, plastics, and pesticides. [10-11-12-13-14]. PAHs can be found almost everywhere in the environment such as air, water, and soil. They are present in the air and transferred to water and soil by precipitation and dust. EPA has identified 16 PAH compounds as major pollutants. These are naphthalene, acenaphthene, acenaphthylene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, chrysene, benzo[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd] pyrene, benzo[ghi]perylene and dibenzo[a,h]anthracene. Waste containing PAHs, which are the main pollutants, also damages living ecosystems. PAHs are hydrocarbon compounds containing two or more benzene rings but no elements other than carbon and hydrogen [15-16].

Smoking is applied to all kinds of meat and meat products, cheese, and all seafood products, including shellfish [17]. Smoked (smoked) fish are products that are salted in a certain procedure in the smoke provided by the wood chips of hardwood trees that shed their leaves in winter, salted with a certain procedure obtained by keeping fresh fish flavored and a prolonged storage period [18]. With smoking, the storage period of the product is considerably extended, and the aroma given to the product by the smoke components gives the product a different aroma. The purpose of smoking technology is to remove some of the water contained in the fish from the meat and to prevent the development of microorganisms by allowing the bactericidal substance in the smoke to pass to the fish. Since the beginning of the use of fire by humans, it has been possible to smoke and dry foodstuffs. However, it is known that modern smoking was first applied to herring in the Middle Ages [19-20]. The purpose of smoking fish and other seafood is to give a different taste, odor, and aroma and to prevent consumption from being monotonous. However, due to the bactericidal and antiseptic effects of smoking on microorganisms, the shelf life of the products is significantly extended. For smoking, the best example of fish is fish with a high fat content. These are sturgeon, trout, salmon, horse mackerel, mackerel, bluefish, sardine, and eel. If lean fish is smoked, the fish is expected to be dry and tough [18-21]. In our country, the consumption habits of smoked seafood products are not at the desired level, but they are preferred by some people who are sensitive to different flavors. The reason for consumption is the different flavors and the aromatic smell of the wood chips used. In other countries, a wide variety of foodstuffs are consumed using smoking technology. In addition to seafood products, foods such as cheese, meat, and chicken are flavored by smoking in the smoke aroma of wood. If the fumigation technology is not applied in a controlled and conscious manner, undesirable compounds occur in the foods to be consumed. These compounds accumulate in the human body and cause serious health problems in humans, including stomach and lung cancer, by showing cancer effect and mutagenic effects. For this reason, smoking should be done in modern ovens rather than the traditional smoking process, which is widely used in our country. Especially with the use of the correct wood species (or wood sawdust) to be used during the smoking process, the rate of PAH compounds in foods will be minimized. Thus, the risk of the consumption of smoked products will be reduced. In this study prepared within this framework; the analyses of trout smoked with olive (*Olea europaea*) wood shavings, pinar (*Quercus aucheri*) wood shavings, and oak (*Quercus Sp.*) wood shavings were carried out by GC/MS device and the results were compared and the compliance of the PAH and other aromatic components

with the limit in the Food Codex was examined and the results were evaluated according to the codex. In addition, it was aimed to determine the effects of PAH pollutants, which are known to be an important threat to human health and are mainly caused by incineration, on aquaculture products consumed by smoking [22].

2. Material and Method

2.1. Collection of Fish and Water Samples

Fresh trout and fumigated trout samples were taken from the fish farms established on the Eşen stream originating from Fethiye-Ören Village, and non-fumigated trout samples were taken from the facilities located in Yaka Village. Subsequently, the amounts of PAHs in these fish samples were analyzed and evaluated to make comparisons.

2.2. Chemical Parameters in Water Samples of Fishes on which Research was Conducted

The chemical parameters in the water samples in which the fish were grown are given below:
* Temperature Determination: Temperature values were measured on-site with a mercury thermometer.
*Conductivity Determination: Conductivity was measured at the sampling site.
* pH Determination: pH was also measured with a different probe of the conductivity measurement system.
*Dissolved Oxygen: A CellOx dissolved oxygen probe was placed, and readings were taken at the sampling site.

2.3. Smoked Trout Processing

In this study, 21 fish with an average weight of 250 grams were selected and the preliminary studies for each parameter are given below respectively.

2.3.1. Cleaning and Preparation Process

The fish to be smoked must first be thoroughly cleaned under pressurized water, and all blood, mucous secretions, scales, etc. must be removed. Afterward, it is cut from the anus to the head, and internal organs and kidneys are removed and cleaned. During cleaning, internal organs, kidneys and mucus layer are washed carefully. Kidney tissue is cleaned thoroughly with a scalpel, etc. The retention of these tissues will cause the fish meat to deteriorate during smoking and then acquire an unpleasant appearance.

2.3.2. Salting Process

The salting process is considered one of the preliminary preparations applied to the fish before smoking and plays an important role in terms of the quality of the final product. It is known that salting has many effects. First, it adds firmness to the fish by removing water from the fish meat and denaturation of its proteins; in certain cases, it helps to stop bacterial activity, thus giving the food a flavor over time. Salt has a very strong structure and gives the fish meat a very attractive appearance. Salt can remove moisture faster than the drying effect. Thus, by removing water from the food, the water activity of the food will be reduced, microorganism activities will be prevented, and the pre-conservation process will be carried out. Salting can be done with a strong salt solution or dry salt.

2.3.3. Drying Process

Fish should usually be thoroughly dried before smoking. Drying is done for 2 reasons. The first is to allow the salt to penetrate evenly and spread well throughout the flesh, and the second is to achieve a glossy and firm coating.

2.3.4. Smoking Process

Smoking is always applied to fresh or frozen fish. The food obtained by smoking stale fish does not look and taste good and will spoil in a very short time. Foods to be smoked, whether fresh or frozen, must be of good quality. Fish to be used fresh should be stored in an icy environment until they are brought to the processing places. Different woods and wood chips are used in smoking. Since the

composition of the various woods is also different, the composition of the smoke will also vary considerably. Many types of fuel are used in smoking, such as animal manure, corn cobs, and all kinds of hard and soft woods. However, as a rule, hardwoods themselves and sawdust are the most suitable materials for smoking. Coniferous trees are not suitable for smoking. This type of wood leaves a lot of soot; on the other hand, it gives off an unpleasant turpentine smell and flavor [19-23]. The use of wood chips instead of wood in the smoking process has also been found to be advantageous. Wood chips ignite more easily than the wood itself and give a very strong fire. As a result of the use of various fuels in smoking, foods with different compositions of smokers and, consequently, different smokiness and flavors are obtained [21]. More than 250 different compounds have been found in wood incense, including aldehydes, ketones, phenolic compounds, organic acids, cresols, formaldehyde, and various polycyclic aromatic hydrocarbons.

Among these, formaldehyde and phenols are the most effective compounds in order of importance [24]. It is known that foods smoked with hard wood, such as hornbeam and oak, taste better than foods smoked with soft wood, such as poplar willow and willow. Generally, the wood species to be used for smoking can be oak, hornbeam, laurel, linden, beech, poplar, and corn cobs. In addition, fruit trees such as apples, cherries, and pears are known to produce pleasant, flavored incense [23-25-26]. The wood chips of the trees in question must be completely dry and passed through a fine-edged saw.

Figure 1. Photo of smoked fish

2.4. Smoked Fish Codes

The codes given for the smoked fish samples to be analyzed in this study presented in Figure 1 are also given in Table 1.

Before the process, the cleaned fish were kept in ice water for 30 minutes and drained. Then they were kept in 20% salt water for 15 minutes and cooked in 40°C-50°C for 30 minutes and 60°C-90°C for 15 minutes. After cooking, it was kept for 45 minutes, and smoke was given. All samples were repeated 3 times, and then the cooked samples were divided into two parts: one part was used for sensory analysis, and the other part was used for extractions.

Table 1. Smoked fish sample codes

Wood Sawdust Type	Skin Part	Skinless Fleshy Part	Skinned Fleshy Part
Olive (*Olea europae*) wood sawdust	ZN-1	ZN-2	ZN-3
Pinar (*Quercus aucheri*) wood sawdust	PN-1	PN-2	PN-3
Oak (*Quercus* sp.) wood sawdust	-	-	MN-2

2.5. Smoked Trout Processing

2.5.1. Extraction

From the smoked fish, especially the skin and the parts close to the skin, 3 g of each sample was weighed into a 50 ml centrifuge tube, and 2 ml of deionized water was added and vortexed (1 minute). Then 10 ml of deionized water was added, 2 ceramic bars were placed, and 15 ml of acetonitrile was added and vortexed for 1 minute. Shaken vigorously with salt mixture (6 g $MgSO_4$ + 1.5 g NaCl) for 2 minutes, then centrifuged at 4000 rpm for 5 minutes. The 8 ml acetonitrile layer was then transferred to a 15 ml AOAC fatty sample d SPE tube and vortexed once more for 1 min (Agilent Bond Elut QuEcheRS fatty sample dispersive SPE 15 ml tube P/ n 5982-5158). Finally, the samples were centrifuged at 4000 rpm for 5 minutes and analyzed by GS/MS.

2.5.2. Pre-enrichment of Extracts in a Rotary Evaporator

Since the samples supplied were approximately 750 mL of solvent, their volume had to be reduced for pre-enrichment. A rotary evaporator was used for this process. The samples were placed in the evaporation flask of the device and enriched under a vacuum of approximately 600 mbar and 40°C water bath conditions until the volume was reduced to 10 mL. After the volume was reduced to 10 mL, the samples were transferred to 15 mL amber vials and stored in a deep freezer at -18°C until analysis. At the end of this stage, clean-up and final enrichment were performed.

2.5.3. GC-MS Parameters

The samples prepared for analysis were analyzed by GC/MS (Agilent 6980 N GC - Agilent 5973 inert MS) to determine their concentrations. A separation and data analysis method were developed to analyze and determine the concentrations of 16 target PAH compounds and 4 surrogate compounds in GC/MS. Using standards for 16 target PAH compounds (Ultra Scientific PM 610) and 4 surrogate compounds (Accu Standard M-525 IS), a calibration was first performed for these compounds, and then the ions of the compounds were grouped and analyzed in 8 separate SIM windows for analysis in "Selected Ion Monitoring" (SIM) mode. GC column 30 m x 250 µm x 0.25 µm nominal film thickness, 5% Phenyl Methyl Siloxane (HP-5MS), capillary column Liner is splitless glass liner (Agilent Technologies) with deactivated glass cotton, Carrier gas: Ultrapure Helium, 99.999%, 1mL/min, Injection type: Splitless, Injection port temperature: 280°C, Oven temperature: 70°C (4 min), 7°C/min to 250°C (5 min), 5°C/min to 300°C (8 min), Injection volume: 1 µL, Mass spectrometer electron impact: 70 eV, Mass spectrometer quadrupole temperature: 150 °C and Mass spectrometer source temperature: 230 °C.

2.5.4. Statistical Evaluation

The results of the experiments will be averaged in 5 parallel experiments, and the confidence interval $p<0.05$ will be accepted. Student *t*-test and ANOVA procedures were applied in all calculations.

3. Results and Discussion

In this study, 21 trout samples were smoked using 3 wood chips.

3.1. Results of Chemical Parameters in Water Samples

The chemical parameters of the water samples in which the fish were raised are given in Table 2. The trout smoked with different wood chips were first divided into two parts for sensory and chemical analyses. While one part was presented to the taste of the panels participating in the sensory analysis, the other part was divided into 3 different parts: skin, skinless meaty part, and skinned meaty part, and homogenized among themselves. To prepare these homogenized samples for chemical analyses, they were extracted with the method given below. Sensory analyses were carried out by a panel of 20 expert personnel from Muğla Sıtkı Koçman University, Faculty of Aquaculture and Food Products Application and Research Center. Appearance, taste, and aroma parameters were selected in the tests.

Table 2. Physicochemical properties of the pond water in which the fish were raised

Wood Sawdust Type	Skin Part	Skinless Fleshy Part	Skinned Fleshy Part
Olive (*Olea europae*) wood sawdust	ZN-1	ZN-2	ZN-3
Pinar (*Quercus aucheri*) wood sawdust	PN-1	PN-2	PN-3
Oak (*Quercus* sp.) wood sawdust	-	-	MN-2

Each panel evaluated 3 products out of 10 points according to these three parameters [27]. According to this, trout smoked with olive wood sawdust was more appreciated than oak and pine wood sawdust; while it was 50% appreciated immediately after smoking, this rate increased to 60% after waiting for 4 hours and to 75% in smoked fish kept for a day. (Table 3) shows the average scores given by the panelists for the 3 selected parameters (each parameter was evaluated out of 100) of smoked fish obtained from olive, oak, and pine wood sawdust as percentages.

3.2. Results of Chemical Parameters in Water Samples

Trout farming, which is an important production and export source for our region, has gained an important momentum in our country. In the trout trade, which provides an important added value for the economy of our country, it is aimed to carry out this research with the conviction that the widespread production of smoked fish, which is a different form of presentation in the market, will provide a new added value. Considering the plant diversity of our country and the smokery resources accepted among the people, olive wood and pinar wood are important natural resources for cooking meat products. In this approach, olive and pine wood chips were investigated as a new source to produce smoked fish. In this approach, olive and pine wood chips were used in addition to oak wood, which is commonly used for smoking, and the results were compared with those of previous products. To date, oak-smoked trout is the best-known in the market. Trout smoked with olive and pine wood chips were compared with smoked fish produced with oak wood chips under the same conditions, both sensory and chemical (PAH and other aromatic compounds). Smoked fish were chemically analyzed in 3 different parameters: skin, skinless flesh, and flesh with skin. According to the results obtained, smoked fish obtained with olive wood sawdust was more accepted in sensory analysis than those produced with Pinar and oak wood sawdust, and 60.1% of the participants of the sensory panel determined that olive wood had a slightly different aroma than the others.

Table 3. Sensory analysis of smoked fish using olive, oak and pine wood shavings

	Trout smoked with olive wood sawdust			**Trout smoked with oak wood sawdust**			**Trout smoked with pinar wood sawdust**		
	Taste	Aroma	Appearance	Taste	Aroma	Appearance	Taste	Aroma	Appearance
Immediately after smoking	150 (%50)			120 (%40)			114 (%38)		
4 hours after smoking	180 (%60)			135 (%45)			126 (%42)		
1 day after smoking	225 (%75)			165 (%55)			150 (%50)		

Extraction of Polycyclic Aromatic Hydrocarbon (PAH) compounds was carried out. For this purpose, 3 g of smoked fish, especially the skin and parts close to the skin, were weighed into a 50 ml

centrifuge tube. 2 ml of deionized water was added and vortexed for 1 minute. Then 10 ml of deionized water was added and divided into 2 ceramic bars, and 15 ml of acetonitrile was added and vortexed again for 1 minute. The salt mixture (6 g $MgSO_4$ + 1.5 g NaCl) was shaken vigorously for 2 minutes, then centrifuged at 4000 rpm for 5 minutes, and the 8 ml acetonitrile layer was transferred to a 15 ml AOAC fatty sample SPE tube, vortexed for 1 minute (Agilent Bond Elut QuEcheRS fatty sample dispersive SPE 15 ml tube P/ n 5982-5158) and centrifuged at 4000 rpm for 5 minutes. The compositions were then analyzed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS) (Agilent 6980 N GC - Agilent 5973 inert MS). A separation and data analysis method were developed to analyze 16 target PAH compounds and 4 surrogate compounds. For this purpose, the compounds targeted for analysis were first calibrated using the standards and then the ions of the compounds were grouped in 8 separate SIM windows for analysis in "Selected Ion Monitoring" (SIM) mode [28]. GC column: 30 m x 250 µm x 0.25 µm nominal film thickness (5% Phenyl Methyl Siloxane) HP 5MS capillary column (Splitless glass liner with liner deactivated glass cotton-Agilent Technologies), Carrier gas: Ultrapure Helium (99.999%, 1mL/min), Injection type: Splitless, Injection port temperature: 280°C, Furnace temperature: gradually 70°C (4 min), 7°C/min to 250°C (5 min) and 5°C/min to 300°C (8 min), Injection volume: 1 µL, Mass spectrometer: Electron impact: 70 eV, Quadrupole temperature: 150 °C and Source temperature: 230 °C.

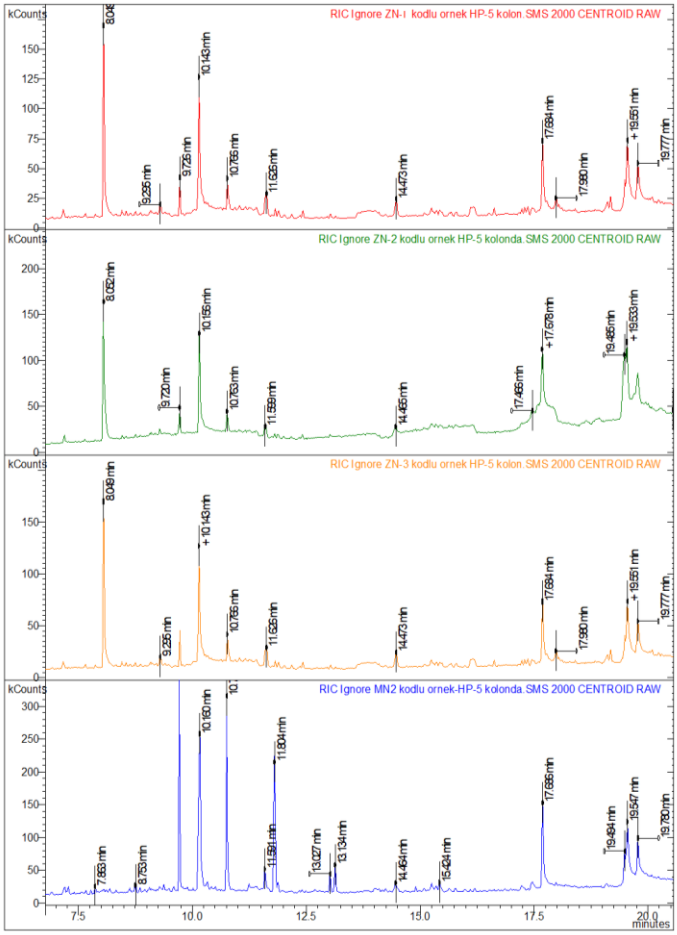

Figure 2. Comparison of GC-MS Chromatograms of lipid phase of trout smoked with olive (ZN-1, ZN-2, ZN-3) and oak (MN-2) wood chips

The mass spectra of each compound were obtained from the chromatograms obtained from the GC/MS system, and the mass spectra of the components were identified using NIST10 and WILEY libraries. In addition, the skin, skinless fleshy part, and fleshy part of the skin of fish smoked with olive wood chips were investigated separately and compared with smoked fish smoked with oak wood, which is widely used (Figure 2). Similarly, chromatograms comparing the relevant parts of trout smoked with pine wood chips and trout smoked with oak wood are given in (Figure 3), as well as GC/MS

chromatograms comparing the fleshy part of trout smoked with olive wood without skin and the fleshy part with skin are given in (Figure 4). According to the results obtained, 14 compounds other than fat components were determined in the skin of trout smoked with olive wood sawdust, 10 compounds were determined in the meaty part without skin, and 13 compounds in the meaty part with skin. Similarly, while 22 compounds were determined in the skin parts of the fish smoked with pine wood sawdust, 21 compounds were determined when the skin and meat parts were evaluated together, and the structure of 5 compounds could be elucidated only in the meat part. The presence of 18 compounds was also found in the samples smoked with oak wood sawdust. As a result of the evaluation of the 7 samples studied, 2,6-Dimethoxy Phenol (syringol), Niacinamide, 1,3-Dimethoxy-5-(1-propenyl)-Benzene-2-acetate, Diisooctyl phthalate and squalene compounds were found to be common in all samples (Table 4).

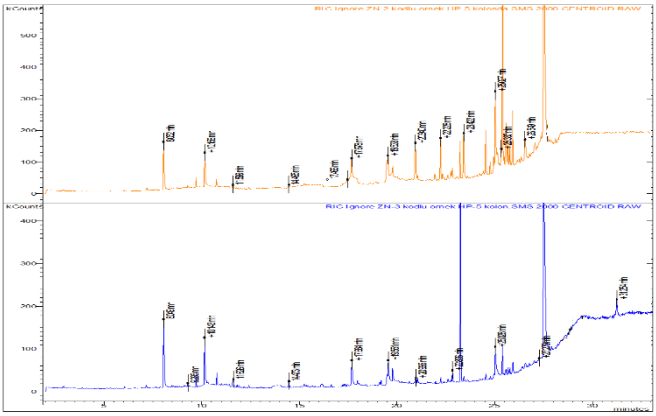

Figure 3. Comparison of GC-MS Chromatograms of the lipid phase of the fleshy area (ZN-2) and the fleshy area including the skin (ZN-3) of trout smoked with olive wood sawdust

When a total of 29 known compounds in 7 samples were analyzed, Benzo[α]pyrene, which is evaluated in the PAH group, was observed as a trace in the skins of fish smoked with olive wood and pine wood sawdust as well as oak sample. Another PAH, 13-(1-Naphthyl)-13H-Dibenzo[a,i]fluorene, was detected at the level of 3 µg/kg in the skin parts of fish smoked with pinar wood sawdust, while it was found at trace level in the skin and fleshy parts of fish smoked with pinar wood and oak wood sawdust.

Trace compounds are compounds below 0.05µg/kg and are not calculated because they are at very low limits and are indicated as trace in Table 3. The amounts of 2 PAH compounds determined in this study comply with the relevant Communiqué of the Food Codex. Therefore, even if these products are consumed with the skin, they are at a consumable level according to the food regulation. Especially squalene, which is an important bioactive in fish meat or fish meat with skin, is seen as a major compound in all products (olive meat portion 140.54 µg/kg).

Niacinamine or Nicotinamide, which is also in the vitamin B group, is the major compound at the 2nd level, and the presence of syringol (2,6-Dimethoxy Phenol), which shows an important antioxidant activity, and 3,5-Dimethoxy acetophenone, another antioxidant compound, as major compounds in smoked fish leads to the belief that these products will provide a functional food for both the extension of shelf life and the consumer. Mass spectra of PAHs and other aromatic compounds found in the lipid fraction of smoked trout were also taken and the results were supported.

In this study, it is understood that trout farming, which is an important production and export source for the region and provides an important added value for the national economy; trout produced with high quality in our clean rivers are offered directly to the market as cleaned and vacuumed products and partially as smoked fish. Although the demand for smoked fish is low in the domestic market, it attracts more attention in foreign markets.

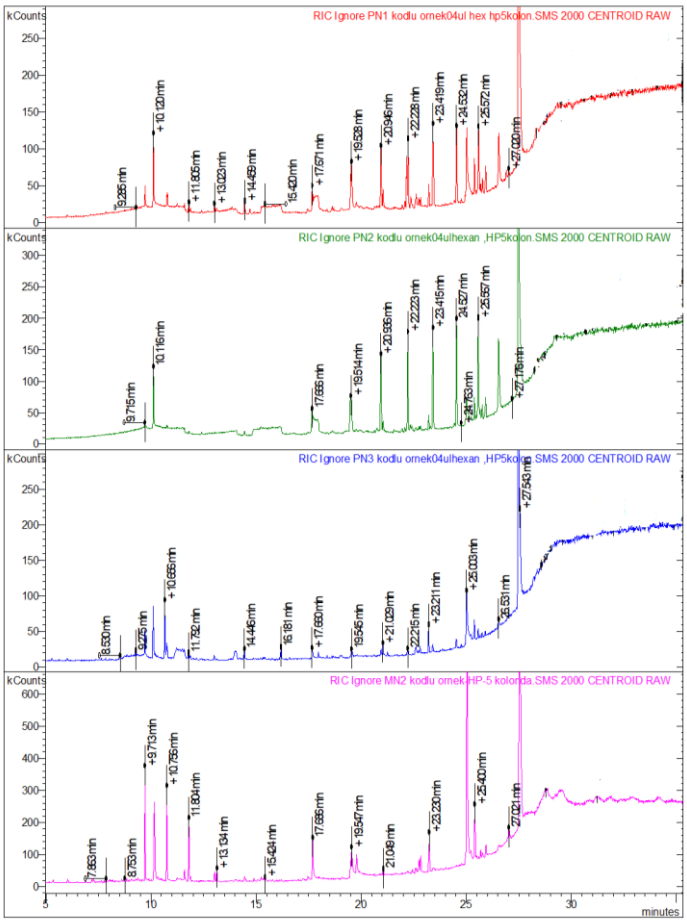

Figure 4. Comparison of GC-MS Chromatograms of lipid phase of trout smoked with pine (PN-1, PN-2, PN-3) and oak wood chips (MN-2)

Since people in the domestic market are not yet sufficiently familiar with the taste and aroma of smoked fish, the consumption of smoked fish in the country lags far behind other countries. In addition to giving the fish a new taste and aroma in smoking, it also provides a very important advantage in extending the shelf life. In this thesis, in addition to oak wood chips, which have been widely used in smoking until today, olive and pine wood chips were used for smoking and smoked fish were compared both sensory and chemically according to wood types.

Among the three different wood shavings subject to this study, olive wood shavings were used for the first time and the comparison of the edible parts of the smoked fish separately was made by us for the first time. According to the results obtained from the sensory analysis, trout smoked with 3 different wood chips were first subjected to sensory analysis and then the components they carry were determined by chemical analysis. According to the sensory analyses, trout smoked with olive wood sawdust was the most popular, with a rate of 60.1%, while oak was the second most popular, with a rate of 46.6%, and trout smoked with pinar wood was the third most popular, with 43.3% (Table 3).

It was stated by all panelists that the aroma and taste of trout smoked with olive wood sawdust was different from other smoked fish. In this respect, it was concluded that a new product with a different taste and aroma could be offered in the smoked fish market with olive wood sawdust.

Table 3. PAH and other aromatic compounds in the lipid fraction of trout smoked with different wood chips

No	RI	Compound name	Trout smoked with olive wood sawdust			Trout smoked with pinar wood sawdust			Trout smoked with oak wood sawdust
			Skin (ZN-1) (µg/kg)	Fleshy Part (ZN-2) (µg/kg)	Skin + Fleshy Part (ZN-3) (µg/kg)	Skin (PN-1) (µg/kg)	Fleshy Part (PN-2) (µg/kg)	Skin + Fleshy Part (PN-3) (µg/kg)	Fleshy Part (MN-2) (µg/kg)
1	1210	1*α*, 9*β*-Dihydro-1H-cyclopropa-[1]- enanthrene	1.38	-	t	-	-	-	-
2	1223	*β*-Hydroxy ethyl phenyl ether (2-Phenoxy ethanol)	37.72	8.11	25.89	-	-	-	t
3	1245	4-Ethyl, 2-methoxy phenol (4-Ethyl quaiacol)	-	-	-	-	-	-	1.46
4	1261	2-Methoxy, 4-vinyl phenol (*p*-Vinilquaiaciol)	2.05	t	1.94	1.90	-	0.45	-
5	1280	2,6-Dimethoxy Phenol (Syringol)	6.32	2.77	5.07	4.87	6.19	8.77	39.46
6	1348	Niacinamide (Nicotinamide)	29.65	17.18	25.93	18.72	15.57	18.74	52.84
7	1375	4-Hydroxy Acetophenone	-	-	-	3.31	-	4.56	-
8	1379	4-Metoxy-3-(methoxymethyl)phenol	9.16	2.58	6.07	0.17	-	t	14.30
10	1381	Isoeugenol	-	-	-	6.07	t	4.56	-
11	1401	2-Hydroxy-5-methoxyacetophenone	-	-	-	0.98	t	1.89	-
12	1430	2,4-Ditertbutyl phenol	-	-	-	2.03	t	1.11	6.65
13	1434	3,5-Ditertbutyl phenol	8.94	2.25	6.04	2.44	-	1.64	31.41
14	1445	3,4,5-Trimethoxytoluene	-	-	-	1.64	-	0.93	t
15	1451	Guaiasil acetone (Methyl vanillil ketone)	-	-	-	0.93	-	t	t
16	1459	3,5-Dimetoksi acetophenone	0.09	t	0.06	-	-	-	-
17	1478	Metoksiöjenol (4-Allil-2,6-dimetoksi phenol)	0.79	-	t	2.24	-	t	4.56
18	1490	*p*-Ethyldiphenyl methane (1-Ethyl-4-benzyl benzene)	t	-	t	1.05	-	t	7.78
19	1587	1,3-Dimethoxy-5-(1-propenyl)-benzene-2-acetate	4.32	1.48	3.53	4.23	t	2.70	2.35
20	1605	1,4-Dimethyl-2-phenoxybenzene	-	-	-	1.44	-	t	-
21	1623	Asetosiringin	-	-	t	-	-	-	t
22	1652	3-Methyl-1,1-diphenyl butane	-	-	-	1.31	-	t	2.65
23	1988	2-Ethylhexyl, *trans*-4-methoxy sinnamate	-	-	-	1.23	-	0.47	-
24	2369	2,2-Methylenebis [6-(1,1-dimethyl ethyl)-4-methyl]-phenol	-	-	-	14.13	-	2.97	-
25	2402	2,4-Bis(1-phenylethyl) phenol	-	-	-	3.56	-	t	-
26	2605	Diisooctyl phthalate	6.02	4.35	5.04	8.68	1.41	5.51	16.63
27	2652	Benzo[α]pyrene	*tr*	-	-	*tr*	-	-	*tr*
28	2710	Squalene	5.12	140.54	115.3	13.46	21.97	6.63	27.57
29	2890	13-(1-Naphthyl)-13H-Dibenzo[a,i]fluoren	-	-	-	3.00	-	t	t

**t*: tres amount; RI: Retention Index on HP-5MS column.

According to the results obtained from chemical analysis; PAHs and other aromatic compounds were investigated by the GC/MS system in 7 different parts obtained from trout smoked with three different wood chips, and it was determined that Benzo[α]pyrene, known as a polycyclic aromatic compound, was found in the skin parts and at trace level (≤0.005 µg/kg), 13-(1-Naphthyl)-13H-Dibenzo[a,i]fluorene was found in the skin or fleshy parts including the skin of the fish smoked with pine and oak wood. When the amounts of PAH compounds are taken into consideration, it is seen that they are suitable according to the Turkish Food Codex and do not pose a risk to the consumer in terms of health, and there is no significant difference in quantitative terms, although the components of smoked trout according to the type of wood sawdust partially change qualitatively. It was determined that nicotinamide varied between 15,57-52,84 µg/kg, and syringol varied between 2,77-39,46 µg/kg depending on the wood type. 2-Phenoxy ethanol ranged between 8.11-37.72 µg/kg in fish smoked with olive wood, while it was not detected in fish smoked with pinar wood. Squalene, together with aromatic compounds in the lipid phase other than PAHs, was found in the range of 5.12-140.54 µg/kg in all samples and 140.54 µg/kg in the skinless part of the trout smoked with olive wood, adding a new functional feature to the trout smoked with olive wood sawdust (Table 4).

In this study, it was determined that olive wood sawdust, which was used for the first time for smoking purposes, can be a new product that is highly appreciated in smoked trout production, and PAH analyses revealed that smoking with wood sawdust is in compliance with the Turkish Food Codex. According to the data obtained, it is expected that fish smoked with olive wood sawdust, which contains higher amounts of Niacylamide, 3,5-Ditertbutyl phenol, 2-Methoxy, 4-vinyl phenol, and especially squalene compared to pine wood sawdust, will be accepted by the consumer in the domestic market both chemically and sensory. In addition, the presence of 2-Phenoxy ethanol compound at the level of 8.11-37.72 µg/kg in olive wood sawdust makes the fish smoked with olive wood different from other wood sawdust. It is thought that 2-Phenoxy ethanol may be the main reason for the different aromas of olive wood sawdust. Considering the results obtained from this study, it is expected that smoking other fish species with olive wood chips will provide an added value in terms of fish exports in the country or offering a new product in the domestic market.

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