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Effects of cooking methods on the formation of heterocyclic aromatic amines of two different species trout

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Abstract

Heterocyclic aromatic amines (HAAs) are sometimes formed in meats and fish cooked at high temperatures. In the present study, the effects of cooking methods by deep-fat frying, pan-frying, grilling and barbecuing on the formation of HAAs of fillets of rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta fario*) were investigated. Barbecued brown trout (1 g) was estimated to contain 0.12 ng of IQ (2-amino-3-methylimidazo[4,5-*f*]quinoline), 0.02 ng 4,8-DiMeIQx (2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline). Grilled rainbow trout (1 g) was estimated to contain 0.02 ng 4,8-DiMeIQx. MeIQ (2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline), MeIQx (2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline) and PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine) were not detectable in all cooked fish.

Keywords: Heterocyclic aromatic amines; *Oncorhynchus mykiss*; *Salmo trutta fario*; Cooking methods

1. Introduction

The formation of heterocyclic aromatic amines (HAAs) has been shown to occur during cooking of protein-rich foods such as meat and fish at temperatures mostly over 150 °C (Knize, Salmon, Mehta, & Felton, 1997; Solyakov, Skog, & Jägerstad, 1999). It has been widely demonstrated that during the cooking of meat HAAs are produced as a result of chemical reactions of creatine, sugars and amino acids, all common components of muscular tissue of animals (Jägerstad et al., 1983; Murkovic, Friedrich, & Pfannhauser, 1997; Salmon, Knize, & Felton, 1997). To date, about 20 carcinogenic/mutagenic HAAs have been isolated and identified in cooked foods (Felton, Jägerstad, Knize, Skog, & Wakabayashi, 2000; Sugimura, 1997).

Factors reported to affect the formation of HAAs in foods include physical factors such as food type, food amount, cooking duration, cooking temperature, cooking equipment and method, pH and water activity with chemical factors such as carbohydrates, free amino acids and creatine. In addition, it was determined that heat and mass transfer, lipid, lipid oxidation and antioxidants have effect on concentration of HAAs (Jägerstad, Skog, Arvidsson, & Solyakov, 1998; Pais, Salmon, Knize, & Felton, 1999).

The aim of this study was to determine the effects of cooking methods (deep-fat frying, pan-frying, grilling and barbecued) on the heterocyclic aromatic amines contents of different species of trout.

2. Materials and methods

2.1. Materials

All chemicals and solvents were HPLC or analytical grade. Water was distilled and additionally purified with activated carbon (Millipore, Bedford, MA, USA). All solutions were passed through a 0.45 μm filter (Milex, MA, USA). Sodium hydroxide was obtained from Fluka (Buchs, Switzerland). Ether, ethyl acetate, hydrochloric acid, methanol and acetic acid were purchased from Riedel-de Haën (Sigma–Aldrich, Seelze, Germany) and acetone, acetonitrile

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and ammonium hydroxide (25%) from Panreac (Barcelona, Spain). Heterocyclic amine standards, IQ (CAS no: 76180-96-6, 2-amino-3-methylimidazo[4,5-*f*]quinoline), MeIQ (CAS no: 77094-11-2, 2-amino-3,4-dimethylimidazo[4,5-*f*]-quinoline), MeIQx (CAS no: 77500-04-0, 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline), PhIP (CAS no: 105650-23-5, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine) and 4,7,8-TriMeIQx (2-amino-3,4,7,8-tetramethylimidazo[4,5-*f*]quinoxaline) were purchased from Toronto Research Chemicals (Downsview, ON, Canada). 4,8-DiMeIQx (CAS no: 95896-78-9, 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline) was kind gift from Dr. Mark G. Knize, Lawrence Livermore National Laboratory, Livermore, CA. Stock standard solutions of 100 μg ml^{-1} in methanol were prepared and used for further dilution. 4,7,8-TriMeIQx was used as internal standard (100 μg ml^{-1} methanolic solution). For the solid phase extraction, diatomaceous earth extraction cartridges (Extrelut-20) and refill material were purchased by Merck (Darmstadt, Germany) and Oasis MCX cartridges (3 cm^3/60 mg) by Waters (Milford, MA, USA). MCX cartridges were preconditioned with ethyl acetate (2 ml).

The fish, rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta fario*) were obtained from Research and Extension Center of Fisheries Department in Agriculture Faculty at Atatürk University, Erzurum, Turkey. Initial weights of trout species were 341.75 ± 26.719 g. On arrival at the laboratory, the fillets were prepared. Weights of trout fillet were 193.89 ± 18.75 g. Then fish were cooked by different cooking methods.

2.2. Cooking of trout

For the deep-fat frying for four fish, fresh sunflower oil (2 l) was used. Whenever the temperature of oil reached 180 °C, the fish were fried for 8 min in a commercial stainless steel deep-fat fryer. The pan-frying process was carried out with a Teflon-coated pan, which was preheated and the surface temperature was measured as 180 °C and then, the fish were fried for 4 min per side without fat or oil. For grilling, hot plate was heated to 180 °C surface temperature, the fish were fried for 4 min on each side on the hot plate with no added fat or oil. For the charcoal barbecued, a bed of charcoal was prepared and ignited. When all flames had subsided, the bed was leveled by raking. The fish were then barbecued over charcoal for 10 min per side. Total cooking time was 20 min. The fish were turned once during frying in the middle of total cooking time in pan-frying, charcoal barbecued and grilling. No salt, oil and flour were applied to fish before and after cooking. Temperatures were measured by using a digital thermocouple (part no. 0560 9260, Testo 926, Lenzkirch, Germany) with surface probe (0603 1992, Testo 926, Lenzkirch, Germany). All experiments were repeated twice. For every replicate, one fish was used for each condition. After the all cooking methods, the cooked fish were cooled at room temperature. Then, the bones and skins of fish were removed. Cooked fish samples were homogenized using a kitchen blender to produce a uniform sample and analyzed to determine proximate composition. Then, fish samples were frozen at −18 °C until analyzed for heterocyclic aromatic amines and were thawed in a refrigerator at 4 °C for 12–24 h prior to use. Freezing was done so that the samples could be stored before clean-up without any risk of further reactions in the fish.

2.3. Chemical analyses

Samples were analyzed for moisture contents, total lipid and pH according to Gökalp, Kaya, Tülek, and Zorba (1999). Total lipid was determined by extraction with petroleum ether. The pH of all samples was measured using a Schott model pH meter (Schott, Lab Star pH, Mainz, Germany).

2.4. Extraction of HAAs

Extraction of HAAs was performed using the method of Messner and Murkovic (2004) which is a modified method originally developed by Gross and Grüter (1992). The use of modified method had some advantages compared with the original method. This method allowed the clean-up in one step and all HAAs were found in only one fraction. According to the method, 1 g sample of cooked fish was dissolved in 12 ml 1 M NaOH. The suspension was homogenized by using a magnetic stirring for 1 h at 500 rpm. The alkaline solution was mixed with 13 g diatomaceous earth (Extrelut NT packaging material, Merck, Darmstadt, Germany) and then poured into empty Extrelut columns. The extractions were made with ethyl acetate and the eluate was passed through the coupled Oasis MCX cartridges. The cartridge was washed with 2 ml 0.1 M HCl and 2 ml MeOH. The analytes were eluted with 2 ml MeOH-concentrated (25%) ammonia (19/1, v/v). The eluted mixtures were evaporated to dryness at 50 °C and the final extracts were dissolved in 100 μl MeOH just before measurement.

2.5. Identification and quantification of HAAs

All samples used in the experiments were purified. HPLC analysis was done once for all 20 samples at the same time. Samples were analyzed on an Agilent 1100 HPLC with UV-DAD dedector (Waldbronn, Germany). Separation of the HAAs was carried out on a reversed phase material (Semi Micro ODS-80 TS column, 5 μm, 250 mm × 2 mm i.d.) from Tosoh Bioscience GmbH (Stuttgart, Germany) with a mobile phase of methanol/acetonitrile/water/acetic acid (8/14/76/2, v/v/v/v) at pH 5.0 (adjusted with ammonium hydroxide 25%) as solvent A and acetonitrile as solvent B. A linear gradient (0% B, 0–12 min; 0–30% B, 12–20 min; 30% B, 20–25 min) was used. Flow rate of the mobile phases was 0.3 ml min^{-1} and the injection volume was 3 μl (an injection program 2.5 μl sample and 0.5 μl internal standard).

Recovery rates for the different HAAs in the fish were determined by the standard addition method. The samples were spiked HAAs mixture at five spiking levels (0.1, 0.3, 0.5, 1 and 2 ng/g freezed fish) by adding different volumes of a methanolic solution of the analytes.

2.6. Statistical analysis

In the present study, a completely randomized design was employed (two replicates), and results were analyzed using SPSS 11.5 (SPSS). Comparison of mean values was made using Duncan test.

3. Results and discussion

The moisture and fat content and pH values of fish (raw and cooked fish) are given in Table 1. The compositions of the raw and cooked trout fish were similar to the findings of other researchers (Gökoglu, Yerlikaya, & Cengiz, 2004; Unlusayin, Kaleli, & Gulyavuz, 2001).

While the raw rainbow trout had a higher ($P < 0.05$) moisture content than cooked by deep-fat frying and barbequing, the fish cooked by the other methods had similar moisture contents. The raw brown trout had a higher ($P < 0.05$) moisture content than those cooked by deep-fat frying and barbecuing. The fish cooked by others methods had similar moisture contents. For rainbow trout, all cooking methods seemed to result in a higher fat proportion, but the difference was statistically significant from others in deep-fat frying method. As a result of cooking fish, since the amount of water decreases, the fat proportion increases in general. Therefore, the fat proportion in cooked fish was higher than that in raw fish. The fat proportion in cooked brown trout was increased compared

Table 1
Means for moisture, fat and pH values of raw and cooked rainbow and brown trout[a,b]

Fish	Condition	Moisture (%)	Fat (%)	pH
Rainbow trout	Raw	72.17 ± 1.10a	3.33 ± 1.24 b	6.43 ± 0.01a
	Deep-fat frying	61.67 ± 1.37c	7.69 ± 0.03a	6.59 ± 0.10a
	Pan-frying	70.27 ± 0.74a	4.29 ± 0.69b	6.51 ± 0.07a
	Grilling	72.45 ± 1.61a	4.18 ± 0.39b	6.74 ± 0.07a
	Barbequing	66.53 ± 0.31b	4.32 ± 0.61b	6.76 ± 0.18a
Brown trout	Raw	74.43 ± 0.11a	2.36 ± 0.02a	6.50 ± 0.01a
	Deep-fat frying	60.54 ± 6.63c	9.31 ± 4.18a	6.56 ± 0.09a
	Pan-frying	71.14 ± 1.26ab	3.83 ± 0.13a	6.53 ± 0.11a
	Grilling	70.59 ± 1.35ab	5.83 ± 2.55a	6.68 ± 0.09a
	Barbequing	64.46 ± 0.67bc	5.89 ± 0.23a	6.60 ± 0.01a

[a] Values are shown as mean ± standard deviation of duplicate.
[b] Within the column values with different letters are significantly different ($P < 0.05$). Values with same letters are not significantly different ($P > 0.05$).

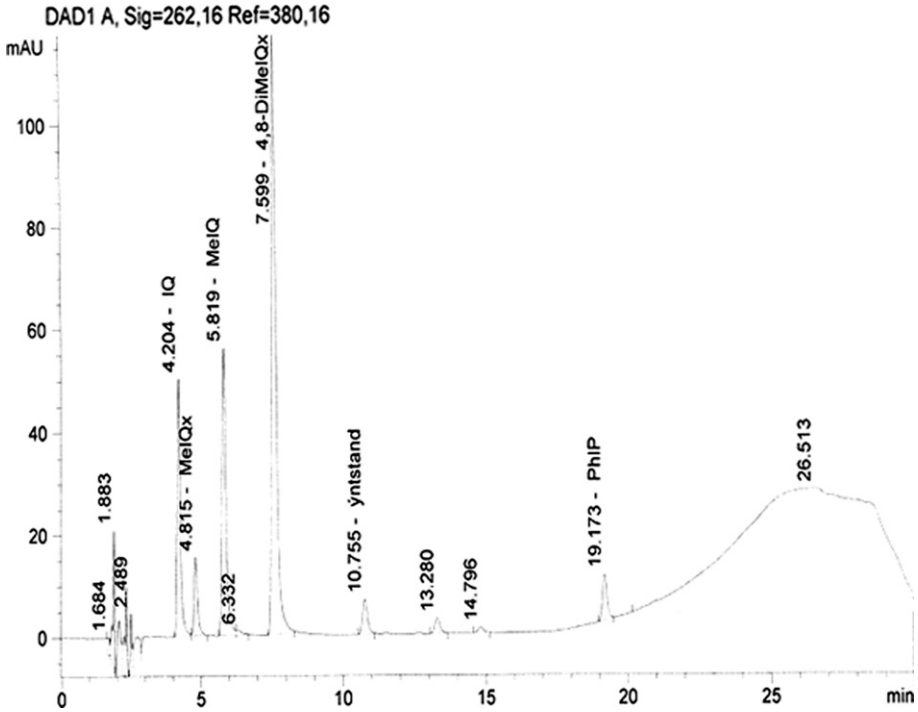

Fig. 1a. HPLC chromatogram from mix stock solutions.

to the raw fish. However, this was not statistically significant ($P > 0.05$). Unlusayin et al. (2001) and Gökoglu et al. (2004) have reported similar findings for rainbow trout. In experiment on brown trout, cooking methods did not significantly ($P > 0.05$) affect fat content of fish. The pH values of both rainbow and brown trout increased upon cooking. However, changes in pH of both raw and cooked rainbow trout and brown trout were not significant ($P > 0.05$).

The presence of five heterocyclic aromatic amines that can be found in cooked meats and that have commonly been studied and reported in the literature was investigated. Fig. 1a shows the HPLC chromatogram from mixed stock solutions. Data from the quantitative HPLC analysis of IQ, MeIQx, MeIQ, 4,8-DiMeIQx and PhIP, expressed in ng/g cooked fish, are presented in Table 2. In this study, one gram of grilled rainbow trout was estimated to contain 0.02 ng 4,8-DiMeIQx. One gram of barbecued brown trout was estimated to contain 0.12 ng of IQ, 0.02 ng 4,8-DiMeIQx (Fig. 1b). MeIQ, MeIQx and PhIP were not detected in any cooked fish samples. The average recovery of the five HAAs was between 40% and 60%. Messner and Murkovic (2004) found a similar recovery of IQ, MeIQx, 4,8-DiMeIQx, PhIP, AαC and MeAαC. Knize et al. (1995) determined average recoveries ranging from 30% to 68% for IQ, MeIQ, MeIQx, 4,8-DiMeIQx, PhIP.

As mentioned above, surprisingly, MeIQx and PhIP, the most abundant heterocyclic amines in cooked food (Nagao, 1999), were not identified in this study. Krone, Yeh, and Iwaoka (1986) have shown that the major mutagens in fried and canned salmon are different from those in fried ground beef. According to Lee and Tsai (1991), macrocomponents such as free amino acids, sugars and creatine were not markedly different in beef, pork, poultry and fish muscles, suggesting that some other minor components are responsible for the different rates and types of

Table 2
Heterocyclic amines (ng/g) in cooked rainbow and brown trout

Fish	Cooking method	IQ	MeIQx	MeIQ	4,8-DiMeIQx	PhIP
Rainbow trout	Deep-fat frying	nd	nd	nd	nd	nd
	Pan-frying	nd	nd	nd	nd	nd
	Grilling	nd	nd	nd	0.02[a]	nd
	Barbequing	nd	nd	nd	nd	nd
Brown trout	Deep-fat frying	nd	nd	nd	nd	nd
	Pan-frying	nd	nd	nd	nd	nd
	Grilling	nd	nd	nd	nd	nd
	Barbequing	0.12[a]	nd	nd	0.02[a]	nd

nd: Not detected.
[a] Detected only one sample.

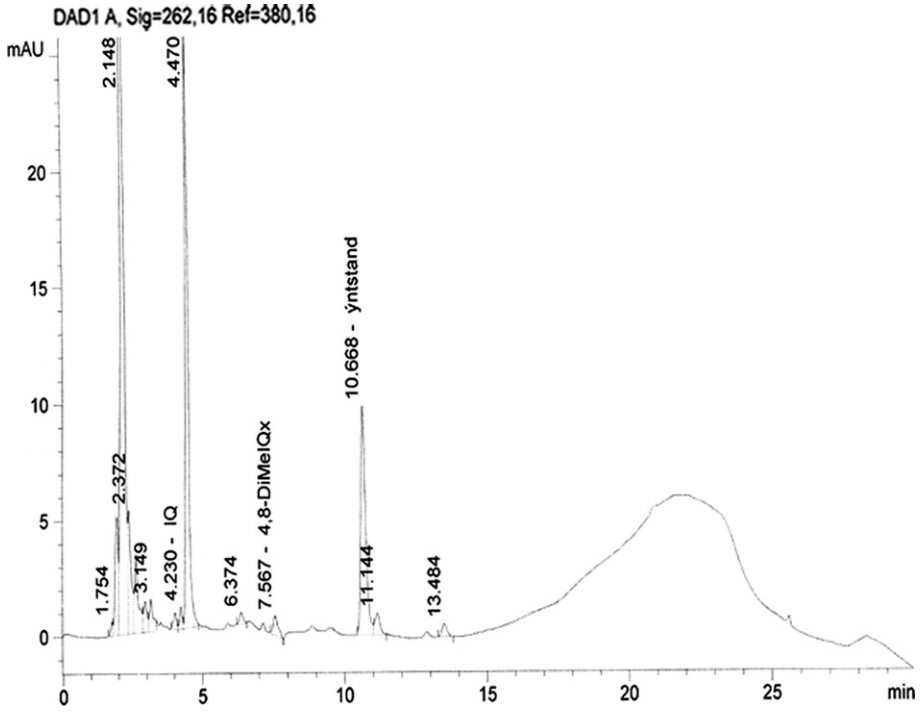

Fig. 1b. HPLC chromatograms from barbequing brown trout.

mutagens formed. The highest concentration of heterocyclic amine in the present study, IQ, was found in the barbecued brown trout. For both fish samples, the total amount of the different heterocyclic amines was below 1 ng/g. The present data on the concentrations of IQ and 4,8-DiMeIQx in rainbow and brown trout are similar to those reported earlier for fish (salmon and Baltic herring), although the cooking methods were different (Gross & Grüter, 1992; Gross et al., 1993; Skog, Augustsson, Steineck, Stenberg, & Jägerstad, 1997).

While broiled salmon flesh has been shown to contain 0.3–1.8 ppb IQ, 0.6–2.8 ppb MeIQ, broiled salmon skin has been shown to contain 1.1–1.7 ppb IQ and 1.5–3.1 MeIQ (Yamaizumi, Kasai, Nishimura, Edmons, & McCloskey, 1986). The same researchers have determined the concentration of IQ and MeIQ as 4.9 and 16.6 ppb in the broiled sardine, respectively. Zhang, Wakabayashi, Liu, Sugimura, and Nagao (1988) found that 1 g of fried walleye pollack, cooked for 16 min at about 260 °C contained 0.16 ng of IQ, 0.03 ng of MeIQ, 6.44 ng of MeIQx, 0.10 ng of 4,8-DiMeIQx and 69.2 ng of PhIP. Lee and Tsai (1991) found that the contents of MeIQx and 7,8-DiMeIQx were 1.1 and 5.3 ng/g of canned roasted eel, respectively. Gross and Grüter (1992) determined that less than 6 min of pan broiling or oven cooking at about 200 °C or barbecuing at 270 °C produced no or low amounts of MeIQx and PhIP. MeIQx and PhIP even showed an apparent decrease with time during pan broiling. Barbecuing of samples for more than 6 min resulted in a tenfold increase in levels of PhIP. MeIQx formation did not follow this trend, and less than 1 ng/g MeIQx was found in such samples, although PhIP were between 0 and 73 ng/g. Wakabayashi et al. (1993) found IQ, MeIQ, MeIQx, 4,8-DiMeIQx and PhIP at 0.16, 0.03, 6.44, 0.10 and 69.2 ng/g in fried codfish, respectively.

Knize et al. (1995) were unable to detect MeIQx, PhIP and DiMeIQx in eighty fast-food fish samples. Skog et al. (1997) have shown that MeIQx and PhIP concentrations are 0.0–0.9 ng/g and 0.02–2.2 ng/g in fried cod fillets, 0.0–0.2 ng/g and 0.07–0.3 ng/g at 150–225 °C in fried Baltic herring, respectively. They have not detected DiMeIQx in fried cod fillets and Baltic herring. Although Pais et al. (1999) found 3.2 ng/g PhIP in fried cod fish at 275 °C for 30 min, they were unable to detect MeIQx. Zimmerli, Rhyn, Zoller, and Schlatter (2001) were unable to detect IQ, MeIQ, MeIQx, 4,8-DiMeIQx and PhIP in household and restaurant type steamed salmon and fried fish sticks and oven roasted fish nuggets.

4. Summary of conclusions

It is rather difficult that the comparison of results in this work with those of other investigators. Also, the results strongly depend on clean-up procedure as well as HPLC detection method used. The content of HAAs in cooked trout is comparably low. This result could be explained by the short cooking time and the low temperatures at the surface of fish and by removing of the skin of fish and also the low fat contents of both rainbow and brown trout.

Fish can be prepared under many different cooking methods and therefore, contained variable levels of heterocyclic aromatic amines. Cooking of rainbow and brown trout by deep-fat, pan-frying, grilling and barbecued had produced no or low amounts of analyzed HAAs. Grilling and barbecuing are other common methods of fish preparation. It is difficult to compare the different cooking methods, because the temperature at the meat surface varies considerably and is difficult to control.

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