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Effects of Cooking Techniques and Levels on the Formation of Heterocyclic Aromatic Amines in Chicken and Fish

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Abstract: The effects of different cooking methods (microwave, oven, hot plate, pan-frying and barbecuing) and levels (rare, medium, well done and very well done) on the formation of heterocyclic aromatic amines (HCAs) in chicken chops and fish fillets were investigated. The cooked samples were analyzed for nine HCAs, including 2-amino-3-methylimidazo [4, 5-f]quinoline (IQ), 2-amino-3-methylimidazo [4, 5-f]quinoxaline (IQx), 2-amino-3, 4-dimethylimidazo [4, 5-f]quinoline (MeIQ), 2-amino-3, 8-dimethylimidazo [4, 5-f]quinoxaline (MeIQx), 2-amino-3, 4, 8-trimethylimidazo [4, 5-f]quinoxaline (4, 8-DiMeIQx), 2-amino-3, 7, 8-trimethylimidazo [4, 5-f]quinoxaline (7, 8-DiMeIQx), 2-amino-1-methyl-6-phenylimidazo [4, 5-b] pyridine (PhIP), 2-amino-9H-pyrido [2, 3-b]indole (AαC) and 2-amino-3-methyl-9H-pyrido [2, 3-b] indole (MeAαC). For chicken samples, while HCAs were only detected in barbecued chicken, it was determined that total HCA amount changed between 3.36 and 8.13 ng g^{-1}. The highest total amounts found in very-well done barbecued chicken chops. It was determined that no HCAs detected in fish fillets cooked with microwave and hot plate. The highest total amounts found in fish for oven, pan-frying and barbecuing were 2.09, 5.89 and 3.52 ng g^{-1}, respectively. AαC and MeAαC were not detected in any samples analyzed.

Key words: Heterocyclic aromatic amines, cooking techniques, cooking level, chicken, rainbow trout (*Oncorhynchus mykiss*), PRS

INTRODUCTION

Heterocyclic Aromatic amines (HCAs) are compounds that are formed naturally during cooking of proteinaceous foods such as meat and fish. Today, >25 various HCAs have been isolated and identified (Sanz Alaejos *et al*., 2008). Epidemiologic studies have shown that most HCAs are highly mutagenic (Felton *et al*., 1984) and almost all of them are also carcinogenic (Sugimura, 1995). The concentrations of HCAs depend on meat type, cooking procedures, pH, water activity, carbohydrates, free amino acids, creatine, heat and mass transfer, lipid, lipid oxidation, antioxidants (Felton *et al*., 1997; Jägerstad *et al*., 1998; Pais *et al*., 1999; Oz and Kaya, 2006; Oz *et al*., 2007). It has been stated that human exposure to HCAs is influenced not only by the type of food and cooking method but also by portion size and intake frequency (Skog, 2004).

The aim of this study was to investigate the effects of different cooking methods (microwave, oven, hot plate, pan-frying and barbecuing) and cooking levels of doneness (rare, medium, well and very well) on the formation of HCAs in chicken chops and fillets of rainbow trout (*Oncorhynchus mykiss*).

MATERIALS AND METHODS

Chemicals and solvents were of High Performance Liquid Chromatography (HPLC) or analytical grade. Water was from a Milli-Q water purification system (Millipore, Bedford, Massachusetts, USA). All solutions were passed through a 0.45 μm filter (Milex, Massachusetts, USA). HCA standards were purchased from Toronto Research Chemicals (Downsview, Ontario, Canada). 4,7,8-TriMeIQx (2-amino-3,4,7,8-tetramethylimidazo [4, 5-f] quinoxaline) was used as the internal standard. The stock standard solutions were prepared according to Oz *et al.* (2007). Chicken chops were obtained from a local market. The fish, rainbow trout (*Oncorhynchus mykiss*), were obtained from Research and Extension Center of Fisheries Department in Agriculture Faculty at Ataturk University, Erzurum, Turkey. The fillets were prepared in laboratory.

Microwave, oven, hot plate, pan-fry and barbecue cooking methods were used in this study. Pre-cooking experiments were done to determine the cooking level of the samples. Cooking level (rare, medium, well and very well) of each sample was determined based on the results of these experiments. The cooking time of the samples cooked with different methods was given according to

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Table 1: Cooking time for the chicken chops and fish fillets (min)

Meat	Cooking methods	Cooking level			
		Rare	Medium	Well	Very well
Chicken	Microwave	3.0	6	9.0	12
	Oven	5.0	10	15.0	20
	Hot plate	5.0	10	15.0	20
	Pan-frying	5.0	10	15.0	20
	Barbecuing	3.0	6	9.0	12
Fish	Microwave	1.0	2	3.0	4
	Oven	3.0	6	9.0	12
	Hot plate	2.0	4	6.0	8
	Pan-frying	2.0	4	6.0	8
	Barbecuing	1.5	3	4.5	6

their cooking levels in Table 1. For the microwave experiment, it was used a kitchen type microwave (Arcelik, Turkey). For microwaving of the samples, cooking was done by automatically selected degrees in microwave. For the oven experiment, it was also used a kitchen type oven (Arcelik, Turkey). For grilling, hot plate was used. The pan-frying process was carried out with a Teflon-coated pan. Before cooking with oven, hot plate and pan-frying, cooking surfaces was preheated to 200°C and then, samples were cooked. 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). For the charcoal barbecued, a bed of charcoal was prepared and ignited. When all flames had subsided, the bed was leveled by raking. No salt, spice, food additive and frying fat or oil were used in cooking procedures. All samples were turned over one a minute during the cooking time. After the cooking, samples were cooled at room temperature and homogenized using a kitchen blender to produce a uniform sample for analyses and frozen at -18°C until analyzed for HCAs. They were thawed in a refrigerator at 4°C for 12-24 h prior to use. The raw chicken and trout samples were analyzed for moisture, total lipids and pH according to Gökalp *et al.* (1999). HCAs however, were only tested in cooked samples.

HCA extraction: HCA content was determined by the method described by the method described by Gross and Gruter (1992) with some modifications. One gram sample of cooked meat was dissolved in 12 mL of 1 M NaOH. The suspension was homogenized by magnetic stirring for 1 h at 500 rpm. The alkaline solution was mixed with 13 g diatomaceous earth and then poured into empty LRC-PRS cartridges. The extractions were made with ethyl acetate and the eluate was passed through coupled diatomaceous earth extraction cartridges. Non-polar HCAs were eluted with 6 mL of 0.01 N HCl, 15 mL MeOH: 0.1 N HCl (60:40) and 2 mL pure water. The eluat in beaker was kept in refrigerator. For the polar HCAs, C_{18} cartridges (100 mg) were coupled with the system and the polar HCAs were eluted with 20 mL ammonium acetate, 2 mL water. The cartridges were dried under nitrogen and then 800 μL of MeOH:NH_3 (9:1) was added and transferred to vials. To continue the non-polar HCAs, 500 μL 32% NH_3 and 25 mL pure water were added to the beaker removed from refrigerator. C_{18} cartridges (500 mg) were coupled with the system and were cleaned with 5 mL MeOH and pure water. Then, the cartridges were dried under nitrogen and then 800 μL of MeOH: NH_3 (9:1) was added and transferred to vials. After polar and non-polar HCAs extraction, 100 μL MeOH was added to vials and vials were stored at -20°C until running.

HPLC analysis: The samples were analyzed on an Agilent 1100 HPLC with UV-DAD detector (Agilent, Waldbronn, Germany). For the analysis of HCAs, a reversed-phase material (Semi Micro ODS-80 TS column, 5 μm, 250×2 mm i.d.) from Tosoh Bioscience GmbH (Stuttgart, Germany) was used with the following HPLC conditions: solvent A was methanol/acetonitrile/water/acetic acid (8/14/76/2, v/v/v/v) at pH 5.0 (adjusted with 25% ammonium hydroxide) and solvent B was acetonitrile. 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. Each injection volume contained 0.5 μL of internal standard. Extraction recoveries were determined by spiking the samples.

Statistical analysis: A completely randomized design has been employed (two replicates) and results have been analyzed using SPSS 11.5 (SPSS).

RESULTS

Moisture, fat and pH values of chicken chops were determined as 74.99±1.26, 3.90±0.66 and 6.40±0.13%, respectively. The same parameters for rainbow trout were 75.93±1.12, 2.58±1.29 and 6.43±0.16%, respectively. The average recoveries of the HCAs varied between 26 and 75% for the PRS method. LOD (limit of detection = 3) and LOQ (limit of quantification = 10) values for this study have been detected as 0.003 and 0.01 ng g^{-1} according to Signal/Noise, respectively.

The HCA content of chicken chops: Data from the quantitative HPLC analysis of the HCAs in chicken extracted according to the PRS method, expressed in ng g^{-1} cooked chicken meat, were presented in Fig. 1. While IQ could not be detected in all chicken samples, including barbecued ones, no HCAs were detected in any samples cooked with microwave, oven, hot-plate and pan-

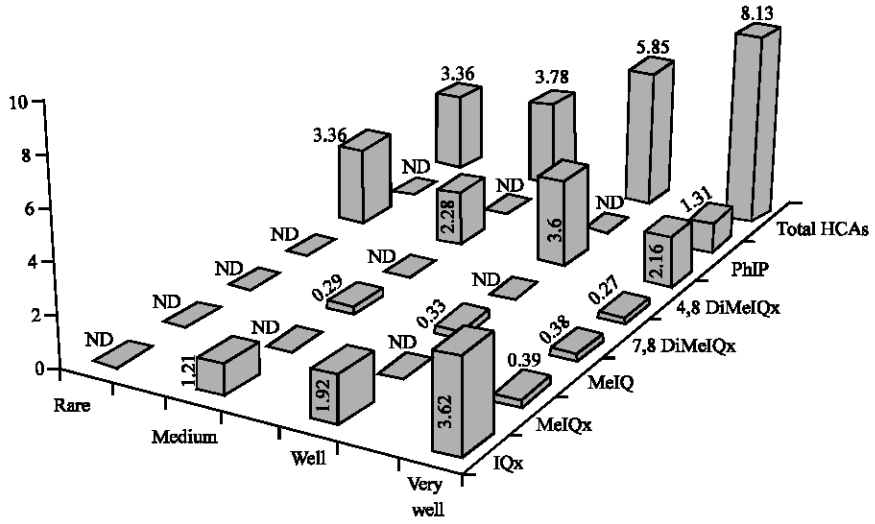

Fig. 1: HCAs in cooked chicken chops (ng g^{-1})

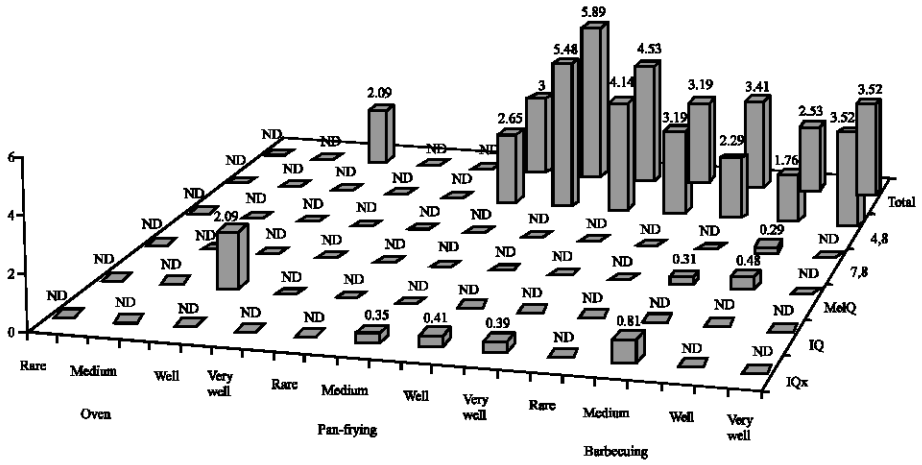

Fig. 2: HCAs in cooked fish fillets (ng g^{-1})

frying. 4,8-DiMeIQx was found in amounts up to around 4 ng g^{-1} in barbecued chicken for all degrees of doneness. However, increasing the cooking level in barbecued samples increased total HCA content. While the difference of the total HCA content between rare and medium cooking levels was minor, the difference of the total HCA content between well done and rare and between very well done and rare level barbecued chicken samples was 1.7 and 2.4 fold, respectively.

The HCA content of rainbow trout: HCA amount in cooked fish fillets extracted according to the PRS method was given in Fig. 2. HCA contents of fish samples cooked with microwave and hot plate at every degree of doneness were undetectable levels. For oven-cooking of fish, 2.09 ng g^{-1} total HCAs amount was only detected in well done cooked samples, all of which was IQ. In the other cooking levels, no HCA could be detected. Although, HCAs could not be detected in rare level pan-fried fish, total HCA amounts for medium, well done and very well done cooking levels reached to 3.0, 5.89 and 4.53 ng g^{-1}, respectively. Barbecuing of fish fillets formed HCAs for all cooking doneness. It was determined that total HCA amount for rare, medium, well done and very well done cooking levels was 3.19, 3.41, 2.53 and 3.52 ng g^{-1}, respectively. In addition, important part of total HCAs amount belonged to 4,8-DiMeIQx compounds (Fig. 2). It is also interesting that MeIQx and PhIP compounds, the most abundant HCAs in meat and fish, could not be detected in rainbow trout. This is not the first time that we

couldn't find this compound not only rainbow trouts but also brown trouts cooked with different techniques (Oz *et al*., 2007).

DISCUSSION

We found that the average recoveries for nine HCAs changed between 26 and 75% for the PRS method. In the studies using the PRS extraction method, Felton *et al.* (1994) and Knize *et al.* (1994, 1995) found that the average recoveries were 26-80, 46-71 and 30-68%, respectively.

The levels of HCAs found in the present study for chicken samples extracted by the PRS method are in the same range with other studies (Tikkanen *et al*., 1996; Skog *et al*., 1997; Solyakov and Skog, 2002; Busquets *et al*., 2004) (IQx and 7,8-DiMeIQx were also detected in the present study). However, the results are quite different with data from Sinha *et al.* (1995) who found that total HCAs (4,8-DiMeIQx, MeIQx and PhIP) of pan-fried, oven-broiled and grilled/barbecued skinless, boneless chicken breasts ranged from 14-77, 6-153 and 27-491 ng g^{-1}, respectively. In contrast to this study, we found that the total HCA amounts of chicken sample were <10 ng g^{-1} for all conditions. The big difference between these results and the results of Sinha *et al.* (1995) is about PhIP. Although, Sinha *et al.* (1995) determined 480 ng g^{-1} PhIP in very well done grilled/barbecued skinless and boneless chicken breasts, we only detected 1.31 ng g^{-1} PhIP in very well done barbecued chicken.

Skog *et al.* (1997) stated that chicken meat roasted at 150-200°C for 30 min contained little or no IQ, MeIQ, MeIQx and 4,8-DiMeIQx and amount of PhIP of the same samples was below 0.03 ng g^{-1}. While no IQ was detected in chicken grilled at 175-200°C for 13 min, below 0.1 ng g^{-1} MeIQ, 0.3 ng g^{-1} MeIQx, 0.4 ng g^{-1} 4,8-DiMeIQx and 2.3 ng g^{-1} PhIP was found by Busquets *et al.* (2004). IQ and MeIQx content of chicken fried 100-200°C for 15 min varied between 0.09 and 0.51 ng g^{-1} and between 0.08 and 0.91 ng g^{-1}, respectively (Chiu *et al*., 1998). While the lowest value for MeIQ, 4,8-DiMeIQx and PhIP compounds was undetectable levels, the highest values for these compounds was 1.21, 0.78 and 2.81 ng g^{-1}, respectively.

It was determined that boiling chicken meat contained no MeIQx, 4,8-DiMeIQx and PhIP and harman and norharman in amounts up to 0.5 ng g^{-1} (Solyakov and Skog, 2002). Same researchers found that deep-fat frying of chicken breasts produced traces of MeIQx, 4,8-DiMeIQx and PhIP. It was also determined that pan-frying of chicken breast contained MeIQx in amounts up to 1.8 ng g^{-1}, 4,8-DiMeIQx in amounts up to 0.6 ng g^{-1} and PhIP in amounts up to 38.2 ng g^{-1}. For oven-cooking, the results are in agreement with results from Solyakov and Skog (2002), who reported that oven-cooking of chicken yielded in general non-detectable amounts of MeIQx and PhIP; however, the samples were not tested for IQx, IQ, MeIQ and 7,8-DiMeIQx. We could not also detected MeIQx and PhIP in oven-cooked chicken. Tikkanen *et al.* (1996) found 0.11 ng g^{-1} MeIQx, 0.13 ng g^{-1} 4,8-DiMeIQx and 4.5 ng g^{-1} PhIP in chicken grilled at 220°C for 40 min. Murkovic *et al.* (1997) detected that the concentration of IQ, MeIQ, MeIQx, 4,8-DiMeIQx and PhIP was 1.1, 0.9, 1.4, 0.4 and 3.8 ng g^{-1} in fried turkey for 20 min.

The present data on the total HCA amount in fish are of the same magnitude as reported earlier for fish samples, although the cooking conditions and fish species are different. Knize *et al.* (1995) were unable to detect MeIQx, PhIP and 4,8-DiMeIQx in eighty fast-food fish samples. Zimmerli *et al.* (2001) were also 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. Yamaizumi *et al.* (1986) determined that IQ and MeIQ content of broiled fish ranged from 0.3 and 1.8 ng g^{-1} and 0.6 and 2.8 ng g^{-1}, respectively. About 0.1 ng g^{-1} MeIQx, 0.03 ng g^{-1} 4,8-DiMeIQx and 1.37 ng g^{-1} PhIP was detected in fried fish by Salmon *et al.* (2006). The highest value of MeIQx and PhIP compounds in barbecued fish was detected as 0.03 and 5.5 ng g^{-1}, respectively by Tikkanen *et al.* (1993).

Oz *et al.* (2007) studied effects of different cooking methods (deep-fat frying, pan-frying, grilling and barbecuing) with various times (4-20 min) on formation of HCAs in rainbow and brown trouts by the Oasis extraction method and found that the highest amounts of IQ and 4,8-DiMeIQx compounds was found as 0.12 and 0.02 ng g^{-1}, respectively. However, the researches could not detect MeIQ, MeIQx and PhIP in any samples.

Zhang *et al.* (1988) found 0.16 ng g^{-1} IQ, 0.03 ng g^{-1} MeIQ, 6.44 ng g^{-1} MeIQx, 0.1 ng g^{-1} 4,8-DiMeIQx and 69.2 ng g^{-1} PhIP in fish fried at 260°C for 16 min. It was determined that MeIQx and PhIP amounts of fish fried at 200°C for 6-24 min varied between 1.4 and 5.0 ng g^{-1} and between 1.7 and 17.0 ng g^{-1}, respectively (Gross and Gruter, 1992). Although, Pais *et al.* (1999) could not detect MeIQx and 4,8-DiMeIQx in fish fried 275°C for 30 min, 3.2 ng g^{-1} PhIP was detected.

CONCLUSION

Many previous studies have shown that cooking methods and time are very important for the formation of heterocyclic aromatic amines. The effects of several cooking methods on HCA levels, including cooking

degrees of doneness, in chicken and fish were investigated by the present study. HCAs are formed generally in cooked meat. No AαC and MeAαC compounds could be detected in any samples studied.

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