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Effects of cooking methods and levels on formation of heterocyclic aromatic amines in chicken and fish with Oasis extraction method

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ABSTRACT

Heterocyclic aromatic amines (HCAs) are potent mutagenic and carcinogenic compounds formed during heat processing of proteinaceous food such as beef, poultry, and fish. The objective of this study was to measure nine HCAs in chicken chops and fish fillets cooked by various methods (microwave, oven, hot plate, pan-frying, and barbecuing) to different degrees of doneness (rare, medium, well done, and very well done). Total HCA amount in chicken changed between 0.24 and 8.21 ng/g, and not only the highest total amount but also the lowest total HCA amount was found in microwave cooked chicken samples. The highest total HCA amount found in fish for microwave, oven, hot plate, pan-frying, and barbecuing were 18.09, 4.28, 3.12, 6.98, and 5.22 ng/g, respectively. The results show that microwave cooking alone is found to possess the highest total HCA amount, followed by pan-frying, and barbecuing of meat samples, and the total HCA amount in cooked samples is low if cooked to rare and medium degrees of doneness. 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) were not detected in any samples.

1. Introduction

Heterocyclic aromatic amines (HCAs) are compounds that are formed naturally during cooking of proteinaceous foods such as meat (Klassen, Lewis, Lau, & Sen, 2002). 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, Skog, Arvidsson, & Solyakov, 1998; Oz, Kaban, & Kaya, 2007; Pais, Salmon, Knize, & Felton, 1999).

Meat is a very important part of our meals contributing valuable nutrients which are beneficial to health. It contains important levels of protein, providing all essential amino acids, vitamins and minerals essential for growth and development. The two most common group of meat are chicken and fish. Chicken and fish can be prepared under a lot of various cooking procedures and thus contain variable levels of HCAs ranging from not detectable levels to hundreds of nanogram per gram of cooked meat. Therefore, it is very important to collect data on levels of HCAs in meat cooked by various methods to different degrees of doneness to minimize the intake of them with our foods.

The relationship between the intake of HCAs with food and cancer risk is still not fully understood (Knize & Felton, 2005). Several epidemiological studies have shown positive correlations between intakes of HCAs with foods and increased risk of various types of human cancer (Sinha et al., 2000; Sinha, Kulldorff, Chow, Denobile, & Rothman, 2001), whereas other studies have not found such correlation (Augustsson, Skog, Jägerstad, Dickman, & Steineck, 1999; Gunter et al., 2005). However, cancers associated with meat consumption can be reduced by changing the cooking methods and levels knowing which one is better for the minimal formation of HCAs regardless of HCAs being human carcinogens or not. Therefore, the amount of these compounds in chicken chops and fish fillets items cooked by various methods to different levels must be established. From the point of estimating the intakes and risks to human health, it is important to quantify HCAs in different meat products prepared in different ways. There are many articles about the influence of cooking methods and levels on HCAs in chicken and fish (Gašperlin, Lukan, Žlender, & Polak, 2009; Oz et al., 2007). However, to our knowledge, the influences of cooking methods including microwave, oven, hot plate, pan fry, and barbecue and cooking levels including rare, medium, well, and very well done have not been investigated in these studies on 9 HCAs in chicken chops and rainbow trouts, *Oncorhynchus mykiss*, at the same time.

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The aim of this study was to determine the influence of different cooking methods (microwave, oven, hot plate, pan-frying, and barbecuing) and degrees of doneness (rare, medium, well, and very well) on the formation of HCAs in chicken chops and fillets of fish, rainbow trout (*O. mykiss*). Since cooking procedures including methods and degrees play an important role in the formation of HCAs, it was hypothesized that there should be a cooking method and level for the less formation in cooked meat.

2. Materials and methods

2.1. Food samples

Chicken chops were bought from a local market with bones, but with no skin. Cooking was performed on the chops with bones. After the removal of the bones, they were analyzed. The fish, rainbow trout (*O. 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. Fish fillets were cooked skin on and they were analyzed after the skin was removed, too.

2.2. Chemicals

All 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). We investigated the presence of 9 heterocyclic amines that can be found in cooked meats and have been commonly studied and reported in the literature. These compounds are 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ, CAS no: 76180-96-6), 2-amino-3-methylimidazo[4,5-*f*]quinoxaline (IQx, CAS no: 108354-47-8), 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline (MeIQ, CAS no: 77094-11-2), 2-amino-3,8-dimethylimidazo[4,5-*f*] quinoxaline (MeIQx, CAS no: 77500-04-0), 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (4,8-DiMeIQx, CAS no: 95896-78-9), 2-amino-3,7,8-trimethylimidazo[4,5-*f*]quinoxaline (7,8-DiMeIQx, CAS no: 92180-79-5), 2-amino-1-methyl-6-phenylimidazo[4,5-*b*] pyridine (PhIP, CAS no: 105650-23-5), 2-amino-9*H*-pyrido[2,3-*b*] indole (AαC, CAS no: 26148-68-5), and 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole (MeAαC, CAS no: 68006-83-7). 2-amino-3,4,7,8-tetramethylimidazo[4,5-*f*] quinoxaline (4,7,8-TriMeIQx, CAS no: 132898-07-8) was used as the internal standard. HCA standards were purchased from Toronto Research Chemicals (Downsview, Ontario, Canada). The stock standard solutions were prepared according to Oz et al. (2007). For the solid phase extraction, an Oasis MCX cartridge (3 cm^3/60 mg, 30 μm) of Waters (Milford, Massachusetts, USA) was used.

2.3. Cooking procedures

Microwave, oven, hot plate, pan-fry, and barbecue as cooking methods have been used in the present 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. Table 1 shows the details of cooking procedures. For the microwave experiment, it was used a kitchen type microwave (Arcelik, Turkey). For the samples to be microwaved, cooking was done at automatically set degrees in microwave. Cooking was performed at 900 W. For the oven experiment, it was also used a kitchen type oven (Arcelik, Turkey). Samples were cooked in special containers which are parts of the microwave and the oven, a glass dish 32.5 cm in diameter, and a pan 36.5 × 45.5 cm, respectively. For grilling, hot plate (Test Lab Equipment, 41.5 × 58.5 cm) 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 all cooking procedures. All samples without microwave and oven cooked were turned over once a minute during the cooking time. Each sample was cooked under the same conditions for all cooking methods, but one after the other. After the cooking, samples were cooled at room temperature and homogenized using a kitchen blender to produce a uniform sample for analyses, and were divided to two pieces for chemical composition and Oasis method. The samples were frozen at −18 °C until analyzed for HCAs. They were thawed in a refrigerator at 4 °C for 12–24 h prior to use.

Table 1
Cooking time for the chicken chops and fish in different cooking methods (min).

Meat	Cooking methods	Cooking level			
		Rare	Medium	Well	Very well
Chicken	Microwave	3	6	9	12
	Oven	5	10	15	20
	Hot plate	5	10	15	20
	Pan-frying	5	10	15	20
	Barbecuing	3	6	9	12
Fish	Microwave	1	2	3	4
	Oven	3	6	9	12
	Hot plate	2	4	6	8
	Pan-frying	2	4	6	8
	Barbecuing	1,5	3	4,5	6

2.4. Chemical analysis

The raw chicken chops and fish fillets were analyzed for water, total lipids, and pH according to Gokalp, Kaya, Tulek, and Zorba (1999). Total lipid was determined by soxhlet extraction with petroleum ether. The pH of samples was measured using a Schott model pH m (Schott, Lab Star pH, Mainz, Germany).

2.5. Extraction of heterocyclic aromatic amines

HCA content was determined by the method described by Messner and Murkovic (2004), with minor modifications, and the samples were analyzed after solid-phase extraction, using an Agilent 1100 HPLC with a diode array dedector (UV-DAD, Agilent, Waldbronn, Germany), as described by Oz et al. (2007). Recovery rates for the different HCAs in the chicken and fish were determined by the standard addition method (Oz et al., 2007).

2.6. Data analysis

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

3. Results and discussion

3.1. Chemical analysis and recoveries

Water, fat, and pH values of chicken chops were determined as 74.99 ± 1.26 g/100 g, 3.90 ± 0.66 g/100 g, and 6.40 ± 0.13,