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# Effects of different cooking methods and fat levels on the formation of heterocyclic aromatic amines in various fishes

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2-Amino-3,4-dimethylimidazo[4,5-f] quinoline (PubChem CID: 62274)
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2-Amino-3,4,8-trimethylimidazo[4,5-f] quinoxaline (PubChem CID: 104739)
2-Amino-1-methyl-6-phenylimidazo[4,5-b] pyridine (PubChem CID: 1530)
2-Amino-9H-pyrido[2,3-b]indole (PubChem CID: 62805)
2-Amino-3-methyl-9H-pyrido[2,3-b]indole (PubChem CID: 62244)

ABSTRACT

Effects of different cooking methods (microwave, dry-heating in a pan, oven, hot plate and barbecuing) and fat levels (lean and fatty) on the formation of heterocyclic aromatic amines (HCAs) of various fishes (salmon, mackerel, sardine, whiting, trout and sea bass) were investigated. Cooking treatment had very significant effect ($p < 0.01$) on water and pH, significant ($p < 0.05$) effect on total HCA content. In addition, pH was very significantly ($p < 0.01$) affected by fish species, while fish species had no significant effect ($p > 0.05$) on total HCA content. The total HCA content of the samples ranged between non-detectable levels and 5.72 ng/g. The highest total HCA content statistically belonged to barbecued samples. In addition, it was determined that the total HCA content of dry-heated salmon and sea bass in a pan was under the limit of detection of the HCAs.



## 1. Introduction

Nutrition is required for maintaining human's life. On the other hand, human has started to pay much more attention to sufficient and balanced nutrition due to developing technology, increasing welfare of families and consumer awareness about nutrition. Therefore, there is continuously increasing demand for food that promotes healthy and good living. According to the report on world fisheries and aquaculture states by FAO, human life is prolonged,

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and people are becoming increasingly richer and more educated eventually becoming more health-conscious in most of the developed countries (FAO, 2012). Today, one of the most common nutritional problems is undoubtedly inadequate intake of animal protein. Between foods of animal origin, fish has a very important place. The benefits of fish on the diet are related to the content of proteins with high biological value and unsaturated fatty acids that is an important advantage in terms of health and vitamins and minerals (Sidhu, 2003). In addition, another important advantage of fish is often more economical compared to red meat and poultry. On the other hand, fish could also contain many harmful substances such as microbial contaminants, toxins or various chemicals (which can contaminate the fish or occur during the processing of fish). Therefore, fish, contributes in the prevention and treatment of certain diseases, and is also responsible for the emergence of certain diseases. Heterocyclic aromatic amines (HCAs) are one of the most important chemicals formed during cooking of fish.

Epidemiologic studies have shown that HCAs are mutagenic and/or carcinogenic compounds that are formed in proteinaceous foods such as meat and fish cooked at temperatures generally above 150 °C (Knize et al., 1995; Sanz Alaejos, Pino, & Afonso, 2008). To date, more than 25 different HCAs have been isolated and identified from foods (Oz & Cakmak, 2016; Oz, Cakmak, Zikirov, Kızıl, & Turhan, 2015; Sanz Alaejos, Ayala, Gonzalez, & Afonso, 2008). The formation and amounts of HCAs are dependent on many factors such as meat type, cooking duration, cooking temperatures, cooking equipment and methods, pH, water activity, carbohydrates, free amino acids, creatine and/or creatinine, heat and mass transfer (Oz & Kaya, 2011a; Oz, Kaban, & Kaya, 2007; Oz, Kaban, & Kaya, 2010; Oz & Yuzer, 2016; Pais, Salmon, Knize, & Felton, 1999; Sanz Alaejos & Afonso, 2011). In addition, the fat content of meat is known to be very important for the formation and the concentrations of HCAs (Jägerstad, Skog, Arvidsson, & Solyakov, 1998; Oz, 2010, 2011; Oz & Kaya, 2011b,c). While the impact of fats on the formation of HCAs has usually been studied in model systems, Robbana-Barnat, Rabache, Rialland, and Fradin (1996) have reported that there is an optimum fat level for the maximum amount of formation of mutagenic compounds and this level is 10% for ground meat. On the other hand, Knize et al. (1985) have found that increasing amount of fat of beef caused an increase in the amount of mutagenic compound and the maximum mutagenicity belonged to beef with 15% fat grilled at 180 °C and 240 °C.

Several papers dealing with the effects of some cooking methods on the formation of HCAs in some fish fillets have been published (Costa et al., 2009; Gross & Grüter, 1992; Iwasaki et al. 2010; Knize et al., 1995; Oz et al., 2007, 2010; Pais et al., 1999; Puangsombat, Gadgil, Houser, Hunt, & Smith, 2012; Richling, Haring, Herderich, & Schreier, 1998; Viegas, Novo, Pinto, Pinho, & Ferreira, 2012; Wong, Su, Knize, Koh, & Seow, 2005; Zhang, Wakabayashi, Liu, Sugimura, & Nagao, 1988). However, to the best of our knowledge, the effect of different fat levels on the formation of HCAs in fish has not been investigated in the literature. The present study was, therefore, undertaken in an attempt to investigate the influences of various cooking methods and fat levels on the formation of HCAs in lean and fatty fishes.

## 2. Materials and methods

### 2.1. Fish fillets preparation

Various fishes (salmon, mackerel, sardine, whiting, trout and sea bass) were obtained from a local fish store, Erzurum, Turkey. Fishes were brought under cold chain to laboratory and the fillets were prepared in laboratory.

### 2.2. Chemicals

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 except HPLC-grade were passed through a 0.45-μm filter (Milex, Massachusetts, USA). IQ, IQx, MeIQ, MeIQx, 4,8-DiMeIQx, 7,8-DiMeIQx, 4,7,8-TriMeIQx, PhIP, AαC and MeAαC were purchased from Toronto Research Chemicals (Downsview, Ontario, Canada). 4,7,8-TriMeIQx was used as internal standard. The stock standard solutions were prepared according to Oz et al. (2015).

### 2.3. Cooking of the fillets

In the present study, microwave, dry-heating in a pan, oven, hot plate and barbecue cooking methods were used, as representative of those most commonly employed domestically and in cuisine for fish. Preliminary cooking trials were done to determine the cooking temperature and duration of the fish samples. All of the cooked samples were edible. 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). To determine the effect of only cooking methods, no salt, spice, food additive and frying fat and/or oil were used in cooking procedures. The cooking processes were done with skinned fish; however, analyses were performed after the skin was removed. All samples except microwave cooked were turned over 1 min during the cooking time.

A kitchen type microwave (Arcelik, Turkey) was used for microwave experiment. For microwaving of the samples, cooking was done by automatically selected degrees for fish in microwave and total cooking time was 4 min. For the dry-heating process of fish fillets, a Teflon-coated pan was used. Cooking surface of the pan was preheated to 180 °C and total cooking time was 8 min. For oven experiment, a kitchen type oven (Arcelik, Turkey) was used. Oven temperature and total cooking time were 180 °C and 10 min, respectively. For grilling, a hot plate (Test Laboratory Equipments, ETC 742, İstanbul, Turkey) was used. Cooking surface of the hot plate was preheated to 180 °C and total cooking time was 8 min. For the charcoal barbecued, a bed of charcoal was prepared and ignited. When all flames had subsided, the bed was levelled by raking. After the cooking, the samples were cooled at room temperature, homogenized by using a kitchen blender (Tefal, Istanbul, Turkey) to have a uniform sample for analyses and were stored at −18 °C for HCA analyses. They were thawed in a refrigerator at 4 °C for 12–24 h prior to use.

### 2.4. Chemical and physicochemical analyses

Water content (hot air oven), crude fat (ether-extraction) contents and pH values of the samples were determined according to Gokalp, Kaya, Tulek, and Zorba (2010). Water content was determined by drying a 5–10 g sample at 105 °C to the constant weight. For crude fat analysis, dried meat samples were extracted with diethyl ether for 8 h pH of the samples was measured using a Schott model pH meter (Schott, Lab Star pH, Mainz, Germany), previously calibrated at pH 4.0 and 7.0.

### 2.5. Extraction of heterocyclic aromatic amines

HCA content of the samples was determined by the method described by Messner and Murkovic (2004), with minor modifications (Oz & Zikirov, 2015). After solid-phase extraction, HCAs were identified and quantified by HPLC (Thermo Ultimate 3000,