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The Effect of Different Thawing Methods on Heterocyclic Aromatic Amine Contents of Beef Chops Cooked by Different Methods

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Abstract: Heterocyclic aromatic amines (HCAs) are formed during cooking of protein-rich foods at temperatures above 150°C. In this study, two different thawing methods (thawing in refrigerate and microwave) were evaluated for HCA contents of beef chops which were cooked by various methods (boiling, oven, microwave, hot plate, pan-frying without fat or oil, pan-frying with oil, and deep-fat frying). Varying levels of 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) (up to 0.45 ng g^{-1}), 2-amino-3-methylimidazo[4,5-f]quinoxaline (IQx) (up to 0.63 ng g^{-1}) and 2-amino-1-methyl-6-phenylimidazo[4,5-b] Pyridine (PhIP) (up to 0.69 ng g^{-1}) were detected in the beef chops by using Ultra Fast Liquid Chromatography (UFLC) while 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-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 analyzed. Cooking methods had a significant ($p<0.01$) effect on the HCA contents of chops. While the HCAs could not be detected in the samples cooked by boiling, oven, microwave and hot plate, HCAs could be detected in samples cooked by deep-fat frying, pan-fry with oil and pan-frying without oil. The highest total amount of HCAs was found 1.77 ng g^{-1} in beef chops which were cooked in pan-fried with oil. Thawing methods (in refrigerator and microwave) did not have a significant effect ($p>0.05$) on the HCA content of the samples. The results show that HCAs can be isolated in a very short time (5 min) by using UFLC.

Key words: Heterocyclic aromatic amines, beef chops, cooking, thawing, UFLC, microvave

INTRODUCTION

Heterocyclic aromatic amines (HCAs) are formed during cooking proteinaceous foods such as meat or fish at temperatures above 150°C (Knize *et al.*, 1997). So far, >25 different mutagenic and/or carcinogenic HCAs have been isolated and identified in foods and/or model systems (Alaejos *et al.*, 2008). The International Agency for Research on Cancer (IARC) regards some of the HCAs as possible human carcinogens (MeIQ, MeIQx, and PhIP as class 2B carcinogens) and one of them as a probable human carcinogen (IQ, class 2A) (Skog, 2004). Concentrations of HCAs can be dependent on meat type, cooking duration, cooking temperatures, cooking equipment and methods, pH, water activity, carbohydrates, free amino acids, creatine, creatinine, heat and mass transfer, lipid, lipid oxidation and antioxidants (Felton *et al.*, 1997; Jagerstad *et al.*, 1998; Pais *et al.*, 1999; Oz and Kaya, 2006; Oz *et al.*, 2007, 2010a-c). 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).

One of the most widely used method of meat preservation is freezing. It retards microbial growth and enzymatic and chemical reactions that cause deterioration and spoilage (Gokalp *et al.*, 1999). Freezing is also done in HCA analyses so that the cooked samples can be stored before clean-up without any risk of further reactions in the meat. Frozen meats should be thawed before extraction. On the other hand, thawing methods may affect the HCA contents of meats. Therefore, the aim of this study was to investigate the effects of two different common thawing methods (in refrigerate and microwave) on HCA contents of the beef chops which were cooked by different methods (boiling, oven, microwave, hot plate, pan-frying without fat or oil, pan-frying with oil and deep-fat frying). Another important aspect of the study is to prove that HCAs can be isolated in a very short time (5 min) by using UFLC instead of HPLC.

MATERIALS AND METHODS

Chemicals and solvents were of High Performance Liquid Chromatography (HPLC) or analytical grade. Water

Table 1: Cooking procedures of the beef chops

Cooking methods	Oil	Temperature (°C)	Time (min)
Pan-frying	-	200	4.5
Pan-frying	+ (40 g)	200	4.5
Deep-fat frying	+ (2 lt)	200	4.5
Boiling	-	<100	20.0
Microwave	-		4.5
Hot Plate	-	200	6.0
Oven	-	200	9.0

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 was used as the internal standard. The stock standard solutions were prepared according to Oz *et al.* (2007). *Longissimus dorsi* muscle used to make chop was obtained from a local slaughterhouse (Et ve Balik Kurumu Et Kombinasi, Erzurum, Turkey).

Cooking procedures of beef chops were shown in Table 1. For boiling, a kitchen type pressure cooker (Tefal, Turkey) was used. For the oven experiment, a kitchen type oven was used (Arcelik, Turkey). For the microwave experiment, again a kitchen type microwave was used (Arcelik, Turkey). Beef chops were cooked at automatically selected temperatures for beef. For grilling, hot plate was used. The pan-frying processes were carried out with a Teflon-coated pan. For the deep-fat frying and pan-frying with oil, fresh sunflower oil was used. Before cooking in oven, hot plate, pan-frying and deep-fat frying, cooking surfaces and media were preheated up to 200°C and then the chops 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). No salt, spice and food additive were used in cooking procedures. Meat samples were turned over once a minute during the cooking time. After the cooking, the cooked samples were cooled at room temperature. The chops were homogenized using a kitchen blender to produce a uniform sample for analyses, then divided into two pieces for refrigerate thawing and microwave thawing and frozen at -18°C until analyzed for heterocyclic aromatic amines. First group samples were thawed in refrigerator at 4°C for 12-24 h and the second group samples were thawed in microwave for 30 sec prior to extraction. The internal temperatures of the samples which were thawed in microwave were about 30°C.

Extraction of HCAs: HCAs were extracted from the samples and purified by using the method described by Messner and Murkovic which is a modified method originally developed by Gross and Gruter (1992).

According to the method 1 g cooked sample was dissolved in 12 mL 1 M NaOH. The suspension was homogenized by using a magnetic stirring for 1 h at 500 rpm at room temperature. 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 by using ethyl acetate and the eluate was passed through coupled Oasis MCX cartridges. The cartridge was washed with 2 mL of 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.

UFLC analysis: Separation of the HCAs was carried out on a Shim-pack XR-ODS (7.5×3 mm, 2.2 μm) from Shimadzu (Shimadzu CO, Kyoto/Japan) 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 (6% B, 0 min; 9% B, 0-1 min; 15% B, 1-3 min; 70% B, 3-4.5 min; 70% B, 4.5-5.5; 6% B, 5.51 min) was used. The flow rate of the mobile phase was 0.9 mL min^{-1} and the injection volume was 3 μL (its 20% was internal standard). The samples were analyzed using a Shimadzu Prominence series UFLC system equipped with an LC-20AD pump, SIL-20ACHT autosampler CTO-20AC column oven, CBM-20A System Controller and SPD-20A UV/VIS detector with a semimicro flowcell (Shimadzu CO, Kyoto/Japan).

The identities of the analite peaks were established by comparing retention times of the analite peaks with standard HCA solutions and also HCA standard spiked samples. Linear regression (nanograms of compound against peak area) was performed for individual HCAs in mix stock solutions. Coefficients of regression line (r^2) for HCA standard curves were 0.9994 for IQ, 0.9960 for IQx, 0.9960 for MeIQ, 0.9993 for MeIQx, 0.9988 for 4,8-DiMeIQx and 7,8-DiMeIQx, 0.9976 for PhIP, 0.9991 for AαC and 0.9989 for MeAαC. Each peak area corresponding to an HCA was expressed as nanograms per gram of cooked meat. Recovery rates for the different HCAs in the chops were determined by the standard addition method. The samples were spiked with HCAs mixture at four spiking levels (0.5, 1, 2, and 2.5 ng g^{-1} freezed meat) by adding different volumes of a methanolic solution of the analytes.

Statistical analysis: In the present study, a completely randomized design has been employed (two replicates).

RESULTS AND DISCUSSION

Recoveries: The obtained recoveries depend on the sample nature and the spiked concentration level. The

average recoveries of the HCAs were 82.3±15, 65.3±14, 103.8±6, 57.8±9, 93.3±20, 100.3±15, 98.3±9, 135.3±20 and 79.8±16% for IQ, IQx, MeIQ, MeIQx, 4,8-DiMeIQx, 7,8-DiMeIQx, PhIP, AαC and MeAαC, respectively. The recoveries for some HCAs (MeIQ, 7,8-DiMeIQx, and AαC) were above 100%. This situation is not valid for only this study (Thiebaud *et al*., 1994). The Limits Of Detection (LOD) and Limits Of Quantification (LOQ) for standard solutions were calculated with a signal to noise ratio of 3 (S/N = 3) and 10 (S/N = 10), respectively. The lowest detected concentrations for each compound within the sample were IQ 0.019 ng g^{-1}, Iqx 0.041 ng g^{-1}, MeIQ 0.063 ng g^{-1}, MeIQx 0.027, 4,8-DiMeIQx 0.019, 7,8-DiMeIQx 0.022, PhIP 0.127 ng g^{-1}, AαC 0.096 and MeAαC 0.074 ng g^{-1}. The lowest quantified concentrations for each compound within the sample were IQ 0.057 ng g^{-1}, IQx 0.123 ng g^{-1}, MeIQ 0.191 ng g^{-1}, MeIQx 0.082, 4,8-DiMeIQx 0.059, 7,8-DiMeIQx 0.069 PhIP 0.386 ng g^{-1}, AαC 0.291 and MeAαC 0.224 ng g^{-1}. Figure 1 shows an UFLC chromatogram of a solution of nine different HCA standards in MeOH (each 10 ng g^{-1}), 4,7,8-TriMeIQx as internal standard and mix stock solution (10 ng g^{-1}).

The HCAs content of beef chops: Beef can be prepared under many different cooking conditions and will thus contain variable levels of HCAs. HCA contents of the samples are shown in Table 2. HCAs could be detected at levels up to 0.69 ng g^{-1} in the samples cooked by pan-frying with or without oil and deep-fat frying. Boiling of beef chops do not lead to the formation of detectable amounts of HCAs, probably because of the low cooking temperature, which does not exceed 100°C. On the other hand, HCAs could not also be detected in oven cooked, microwave cooked and grilled on hot plate beef samples.

The similar results were obtained by Barrington *et al.* (1990), Berg *et al.* (1990) and Oz *et al.* (2010a, b). However, it was reported that cooking methods such as roasting and baking produce low or intermediate levels of

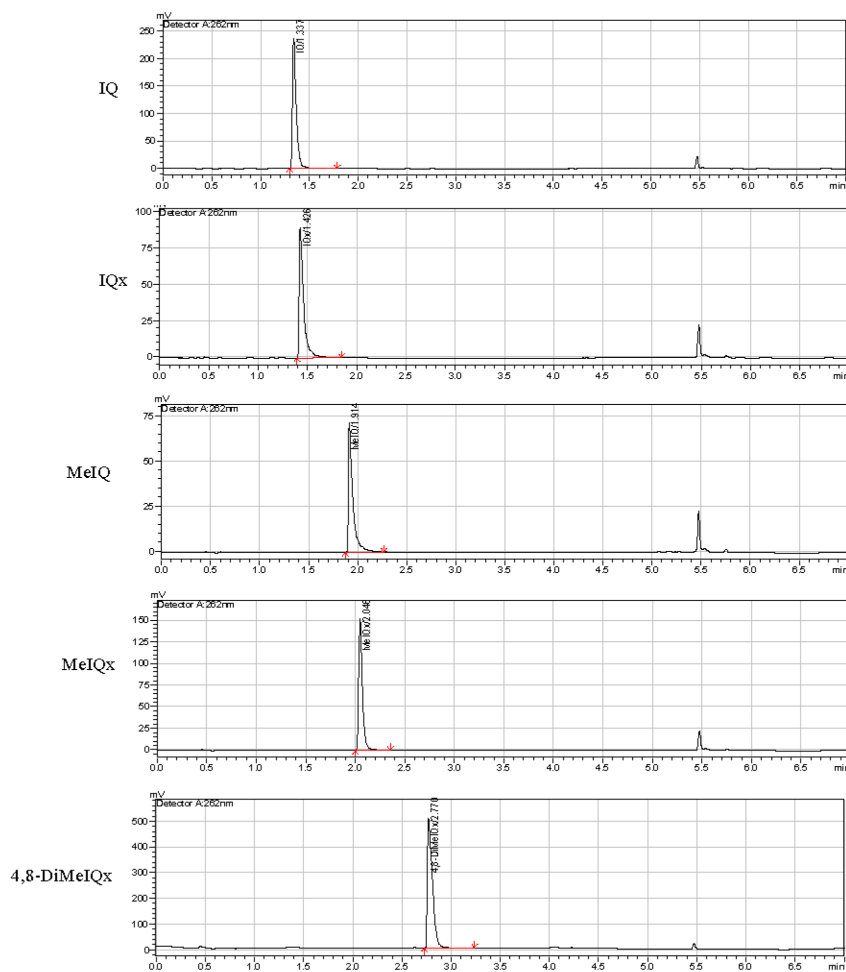

Fig. 1: Continued

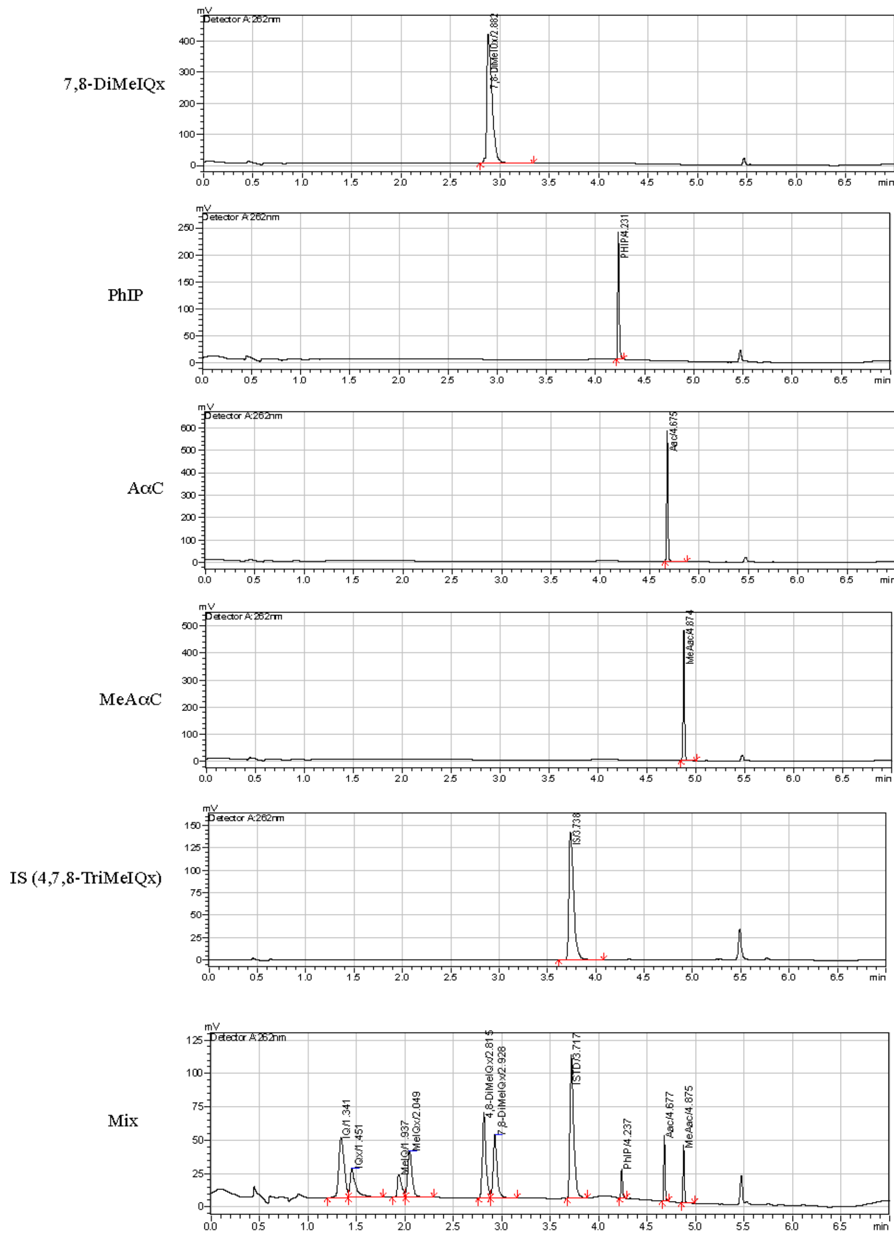

Fig. 1: UFLC chromatogram of a solution of nine different HCA standards in MeOH (each 10 ng g^{-1}), 4,7,8-TriMeIQx as internal standard and mix stock solution (10 ng g^{-1})

HCAs in most protein-rich foods (Bjeldanes *et al.*, 1982a, b). Using oil in pan-frying cooking experiment increased the total HCA amount of the samples. In the study, PhIP was only detected in these samples. IQ and IQx contents of the samples which were cooked both pan-frying without oil and pan-frying with oil had statistically ($p<0.01$) much more those of deep-fat fried samples. Similar results were obtained by Nilsson *et al.* (1986). They reported that using added fats (butter, margarine, or oils) in cooking dramatically increased the amounts of mutagenic compounds.

The results showed that thawing methods (refrigerator and microwave) did not have a significant effect ($p>0.05$) on the HCA content of the samples. Berg *et al.* (1990) reported that reheating or keeping food warm did not alter the amount of mutagenic agents present.

Gross *et al.* (1989) measured that highest total HCA (IQ, 4,8-DiMeIQx, MeIQx and PhIP) amount in beef fried for 10 min at 250°C was 2.6 ng g^{-1}. Total HCA (MeIQx, PhIP and 4,8-DiMeIQx) amount was found as 29.9 and 58.8 ng g^{-1} in pan-fried beef steak at 190°C for 6 and

Table 2: The HCA contents of beef chops (ng g^{-1})

Cooking methods	IQ	IQx	PhIP	Total HCA
Boiling	ND	ND	ND	ND
Oven	ND	ND	ND	ND
Microwave	ND	ND	ND	ND
Hot plate	ND	ND	ND	ND
Pan-frying without oil	0.35±0.03[a]	0.53±0.07[a]	ND	0.88
Pan-frying with oil	0.45±0.03[a]	0.63±0.07[a]	0.69±0.03	1.77
Deep-fat frying	0.09±0.03[b]	0.15±0.07[b]	ND	0.24
Sig.	**	**	**	
Thawing methods				
Refrigerator	0.12±0.02	0.19±0.04	ND	0.31
Microwave	0.13±0.02	0.18±0.04	ND	0.31
Sig.	NS	NS	NS	

**: p<0.01, ND: Not Detected, Sig: Significance, NS: Not Significant. Within the column values with different letters are significantly different (p<0.05)

13 min, respectively (Gross, 1990). Murkovic and Pfannhauser (2000) reported that amount of MeIQx, IQ, 4,8-DiMeIQx and PhIP increased with cooking temperature and time. Pais *et al.* (2000) reported total HCA values (MeIQx, DiMeIQx and PhIP) in well-done beef chops to be 3.0 and 2.8 ng g^{-1} in pork chops. Their values ranged from 9.77 to 185 ng g^{-1} in chops depending on the restaurants that the samples were taken from and the types of muscle that the chops were taken from. Skog *et al.* (1997) determined that total HCA content was <1 and 2 ng g^{-1} in various meat samples cooked at 150 and 175°C, respectively.

Murkovic *et al.* (1998) found a total amount of 33.4 ng g^{-1} for HCAs (10.2 ng g^{-1} IQ, 2.46 ng g^{-1} MeIQ, 13.2 ng g^{-1} MeIQx, 2.26 ng g^{-1} 4,8-DiMeIQx and 5.28 ng g^{-1} PhIP) in beef fried at up to 180°C for 20 min. Felton *et al.* (1994) reported that HCA content of beef fried 200-250°C for 12 min varied between none and 1 ng g^{-1} for IQ, none and 5.1 ng g^{-1} for MeIQx, 0.1 and 1.2 ng g^{-1} for 4,8-DiMeIQx and 0.7 and 13.3 ng g^{-1} for PhIP. Johansson and Jagerstad (1994) could not detect IQ, MeIQ and PhIP in beef barbecued for 10 min but 1 ng g^{-1} MeIQx and 0.2 ng g^{-1} 4,8-DiMeIQx were found in the same samples. In another barbecuing study, MeIQx and PhIP content varied between 0.2 and 1.8 ng g^{-1} and between 1.8 and 18.4 ng g^{-1}, respectively (Knize *et al.*, 1998). However, IQ, MeIQ and 4,8-DiMeIQx content were found below the detectable levels by the same researches. Although no IQ and MeIQ were detected in beef fried at 175-200°C for 11.2 min, 0.7 ng g^{-1} MeIQx and 0.6 ng g^{-1} PhIP were found (Busquets *et al.*, 2004).

It is difficult to compare the results of the present study with those in related literature when several variables such as the number of HCAs, size of chops and cooking procedures are considered. However, there is a consistency between the results. In addition, liquid chromatography was used in most of the studies for determination of HCA in meat products in the literature.

However, the time required to detect HCA was around 30 min (Felton *et al.*, 1994; Oz *et al.*, 2007, 2010a-c) or longer (Gross and Gruter, 1992; Thiebaud *et al.*, 1994; Knize *et al.*, 1997; Pais *et al.*, 1999). On the contrary, it is notable that nine HCAs could be detected in a very short time (5 min) in this study.

CONCLUSION

In conclusion, boiling, oven cooking, microwave cooking and cooking on hot plate of beef chops are healthier than pan-frying with or without oil and deep-fat frying in terms of the formation of HCAs. In addition, refrigerator and microwave for thawing of the samples do not lead to any difference in the amount of HCAs formed when the meat is subjected to the same temperature, length of cooking time and cooking methods. In the present study, no MeIQ, MeIQx, 4,8-DiMeIQx, 7,8-DiMeIQx, AαC and MeAαC have been detected in the samples analzed. Another important aspect of the present study is to decrease isolation time from about 30 min with HPLC to 5 min by using UFLC. As a result, fewer HPLC-grade solutions are used. Thus, it is more economical to use UFLC instead of HPLC in the analysis of HCAs and it is less harmful for our environment.

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