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Heterocyclic Aromatic Amine Contents of Beef and Lamb Chops Cooked by Different Methods to Varying Levels

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Abstract: Heterocyclic Aromatic amines (HCAs) are formed during cooking of meat at temperatures above 150°C. In this study, different cooking methods (microwave, oven, hot plate, pan-frying and barbecuing) and levels (rare, medium, well-done and very well-done) were evaluated for HCAs content in beef and lamb chops. The HCAs were detected in barbecued and pan-fried beef samples. 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). Results showed that the HCAs could not be detected in samples cooked with microwave, oven and hot plate to all various doneness degrees. The highest total amount of HCAs was found 9.78 and 3.06 ng g^{-1} in beef and lamb chops, respectively. No AαC and MeAαC were detected in any samples analyzed.

Key words: Heterocyclic aromatic amines, beef chops, lamb chops, cooking, solid phase extraction, HPLC

INTRODUCTION

Heterocyclic Aromatic amines (HCAs) are chemical compounds that are formed during cooking proteinaceous foods such as meat or fish, at temperatures above 150°C (Knize *et al*., 1997a). To date, >25 different mutagenic and/or carcinogenic HCAs have been isolated and identified in foods and/or model systems (Alaejos *et al*., 2008). In comparison to other known food mutagens, HCAs are over 100 fold more mutagenic than aflatoxin B_1 and over 2000 fold more mutagenic than benzo [a] pyrene (Warzecha *et al*., 2004). 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 depend on meat type, cooking duration, cooking temperatures, cooking equipment and methods, pH, water activity, carbohydrates, free amino acids and creatine (Felton *et al*., 1997; Pais *et al*., 1999; Oz and Kaya, 2006; Oz *et al*., 2007). In addition, it has been determined that heat and mass transfer, lipid, lipid oxidation and antioxidants have some effect on the accumulation of HCAs (Jagerstad *et al*., 1998). 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 levels (rare, medium, well and very well) on the formation of HCAs in beef and lamb chops.

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). Total 4, 7, 8-TriMeIQx was used as the internal standard. The stock standard solutions were prepared according to Oz *et al*. (2007). Muscles of *Longissimus dorsi* used for both beef and lamb chops were obtained from a local market. Pre-cooking experiments were done to determine the cooking level of the samples. Based on the results of these experiments, the cooking time of the chops cooked

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with different methods was given according to their cooking levels in Table 1. For the microwave experiment, a kitchen type microwave was used (Arcelik, Turkey). Beef and lamb chops were cooked at automatically selected temperatures for beef and lamb, respectively. For the oven experiment, again a kitchen type oven was used (Arcelik, Turkey). For grilling, hot plate was used. The pan-frying process was carried out with a Teflon-coated pan. Before cooking in oven, hot plate and pan-frying, cooking surfaces 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). For the charcoal barbecue, 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 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 and frozen at -18°C until analyzed for heterocyclic aromatic amines. They were thawed in a refrigerator at 4°C for 12-24 h prior to use. The raw samples were analyzed for moisture, total lipids and pH according to Gokalp *et al.* (1999). HCAs, however were only tested in cooked samples.

Extraction of HCAs: 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 M HCl, 15 mL MeOH: 0.1 M HCl (60:40) and 2 mL pure water.

The eluate 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 20 mL ammonium acetate and then 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: In the present study, a completely randomized design has been employed (two replicates).

RESULTS AND DISCUSSION

Table 2 shows the percentage of the average moisture, fat and pH values of raw meats. It was determined by Oz (2006) that the moisture content, fat% and pH values of the muscle of *L. dorsi* varied between 74.69-75.51, 1.26-2.48 and 5.57-5.61%, respectively.

The average recoveries of the HCAs varied between 26 and 75% for the PRS method. In the studies using PRS

Table 1: Cooking time for the beef and lamb chops in different cooking methods (min)

Meat	Cooking methods	Cooking level: Rare	Medium	Well	Very well
Beef	Microwave	1.5	3	4.5	6
	Oven	3.0	6	9.0	12
	Hot plate	2.0	4	6.0	8
	Pan-frying	1.5	3	4.5	6
	Barbecuing	1.5	3	4.5	6
Lamb	Microwave	1.5	3	4.5	6
	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

Table 2: The moisture, fat percentage and pH values of raw meat samples

Meat	Moisture (%)	Fat (%)	pH
Beef chop	71.51±1.49	4.65±0.16	5.47±0.02
Lamb chop	73.96±0.35	6.14±0.81	6.12±0.02

Table 3: HCAs in beef chops (ng g^{-1})

Cooking methods	Cooking level	IQx	IQ	MeIQx	MeIQ	7, 8-DiMeIQx	4, 8-DiMeIQx	PhIP	Total HCAs
Pan-frying	Rare	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]
	Medium	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]
	Well	0.36	0.56	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]	0.92
	Very well	0.61	0.68	0.35	ND[a]	ND[a]	ND[a]	ND[a]	1.64
Barbecuing	Rare	1.11	ND[a]	ND[a]	ND[a]	ND[a]	1.23	ND[a]	2.34
	Medium	3.64	0.49	0.45	ND[a]	ND[a]	1.59	3.61	9.78
	Well	3.65	0.65	0.65	0.28	0.28	0.10	1.21	6.82
	Very well	ND[a]	0.78	0.62	0.31	0.28	ND[a]	ND[a]	1.99

ND[a]: Not Detected

Table 4: HCAs in lamb chops (ng g^{-1})

Cooking methods	Cooking level	IQx	IQ	MeIQx	MeIQ	7, 8-DiMeIQx	4, 8-DiMeIQx	PhIP	Total HCAs
Pan-frying	Rare	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]
	Medium	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]
	Well	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]
	Very well	ND[a]	0.62	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]	0.62
Barbecuing	Rare	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]	1.78	ND[a]	1.78
	Medium	ND[a]	1.28	ND[a]	ND[a]	ND[a]	1.71	ND[a]	2.99
	Well	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]	1.38	ND[a]	1.38
	Very well	ND[a]	0.87	0.38	0.29	0.29	0.19	1.04	3.06

ND[a]: Not Detected

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. LOD (Limit Of Detection = 3) and LOQ (Limit Of Quantification = 10) values for the present study have been detected as 0.003 and 0.01 ng g^{-1} according to Signal/Noise, respectively.

The HCAs content of beef chops: HCA amounts of the samples, extracted according to the PRS method are shown in Table 3. HCAs were not detected in chops microwave-cooked, oven and hot plate cooked for no cooking levels and in chops pan-fried for only rare and medium cooked levels. However, the total HCA amount increased with increasing degree of doneness. Total HCA amounts were detected as 0.92 and 1.64 ng g^{-1} for well and very well-done pan-fried samples, respectively. On the other hand, the total HCA amount of barbecued samples increased until to medium cooking and then gradually decreased. The highest total HCAs amount was detected as 9.78 ng g^{-1} in medium barbecued chops (Table 3).

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 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 9.77-185 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).

The HCAs content of lamb chops: Table 4 shows the HCA content of lamb chops. HCAs could not be detected in lamb chops cooked in microwave, oven and hot plate for all cooking levels like in beef samples. No HCAs could be

detected in pan-fried lamb at rare, medium and well-done levels. However, the total HCA amount of 0.62 ng g^{-1}, all of which was IQ was found in very well-done pan-fried lamb samples (Table 4). The total HCA content of barbecued lamb chops for rare, medium, well-done and very well-done was 1.78, 2.99, 1.38 and 3.06 ng g^{-1}, respectively (Table 4). It was determined that the highest total HCA amounts in lamb chops belonged to very well-done barbecued samples. Although, IQ and MeIQ could not be detected in lamb grilled at 175-200°C for 11 min, 1.3 ng g^{-1} MeIQx, 1.8 ng g^{-1} 4, 8-DiMeIQx and 5.8 ng g^{-1} PhIP were found by Busquets *et al.* (2004).

Skog *et al.* (1997) determined that the maximum content of MeIQx, 4, 8-DiMeIQx and PhIP of lamb meat fried at 150-225°C for 9 min was 0.4, 0.6 and 1.5 ng g^{-1}, respectively and could not detect IQ and MeIQ in any lamb samples. Wakabayashi *et al.* (1993) could not detect IQ and MeIQ in grilled mutton, too. However, they found 1.01 ng g^{-1} MeIQx, 0.67 ng g^{-1} 4, 8 DiMeIQx and 42.5 ng g^{-1} PhIP. Knize *et al.* (1997b) detected 1.6 ng g^{-1} MeIQx and 11 ng g^{-1} PhIP in grilled lamb.

CONCLUSION

In conclusion, pan-frying and barbecuing of samples are more effective than the other cooking methods in terms of the formation of HCAs. In the present study, no AαC and MeAαC have been detected in the samples.

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REFERENCES

Alaejos, M.S., J.H. Ayala, V. González and A.M. Afonso, 2008. Analytical methods applies to the determination of heterocyclic aromatic amines in foods. J. Chrom. B, 862: 15-42.

Busquets, R., M. Bordas, F. Toribio, L. Puignou and M.T. Galceran, 2004. Occurrence of heterocyclic amines in several home-cooked meat dishes of the Spanish diet. J. Chrom. B, 802: 79-86.

Felton, J.S., E. Fultz, F.A. Dolbeare and M.G. Knize, 1994. Reduction of heterocyclic aromatic amine mutagens/carcinogens in fried beef patties by microwave pretreatment. Food Chem. Toxicol., 32: 897-903.

Felton, J.S., M.A. Malfatti, M.G. Knize, C.P. Salmon, E.C. Hopmans and R.W. Wu, 1997. Health risks of heterocyclic amines. Mutat. Res., 376: 37-41.

Gross, G.A. and A. Gruter, 1992. Quantification of mutagenic/carcinogenic heterocyclic aromatic amines in food products. J. Chromatogr. A, 592: 271-278.

Gross, G.A., 1990. Simple methods for quantifying mutagenic heterocyclic aromatic amines in food products. Gross Carcinogenesis, 11: 1597-1603.

Gross, G.A., G. Philippossian and H.U. Aeschbacher, 1989. An efficient and convenient method for the purification of mutagenic heterocyclic amines in heated meat products. Carcinogenesis, 10: 1175-1182.

Gokalp, H.Y., M. Kaya, Y. Tülek and Ö. Zorba, 1999. Practical laboratory guide and quality control in meat and meat products. Atatürk Üniversitesi, Ziraat Fakültesi Yayinlari, pp: 71-99.

Johansson, M.A.E. and M. Jagerstad, 1994. Occurrence of mutagenic/carcinogenic heterocyclic amines in meat and fish products, including pan residues, prepared under domestic conditions. Carcinogenesis, 15: 1511-1518.

Jagerstad, M., K. Skog, P. Arvidsson and A. Solyakov, 1998. Chemistry, formation and occurence of genetoxic heterocyclic amines identified in model systems and cooked foods. Z. Lebensm. Unters Forsch. A, 207: 419-427.

Knize, M.G., C.P. Salmon, E.C. Hopmans and J.S. Felton, 1997a. Analysis of foods for heterocyclic aromatic amine carcinogens by solid-phase extraction and high-performance liquid chromatography. J. Chrom. A, 763: 179-185.

Knize, M.G., C.P. Salmon, S.S. Mehta and J.S. Felton, 1997b. Analysis of cooked muscle meats for heterocyclic aromatic amine carcinogens. Mutat. Res., 376: 129-134.

Knize, M.G., F.A. Dolbeare, K.L. Carroll, D.H. Moore and J.S. Felton, 1994. Effect of cooking time and temperature on the heterocyclic amine content of fried beef patties. Food Chem. Toxicol., 32: 595-603.

Knize, M.G., R. Sinha, E.D. Brown, C.P. Salmon, O.A. Levander, J.S. Felton and N. Rothman, 1998. Heterocyclic amine content in restaurant-cooked hamburgers, steaks, ribs and chicken. J. Agric. Food Chem., 46: 4648-4651.

Knize, M.G., R. Sinha, N. Rothman, E.D. Brown and C.P. Salmon *et al.*, 1995. Heterocyclic amine content in fast-food meat products. Food Chem. Toxicol., 33: 545-551.

Murkovic, M. and W. Pfannhauser, 2000. Analysis of the carcinogenic heterocyclic aromatic amines in fried meat. Fr. J. Anal. Chem., 366: 375-378.

Murkovic, M., D. Steinberger and W. Pfannhauser, 1998. Antioxidant spices reduce the formation of heterocyclic amines in fried meat. Zeitschrift Lebensmitteluntersuchung Forschung A, 207: 477-480.

Oz, F. and M. Kaya, 2006. Mutagenic and carcinogenic heterocyclic aromatic amines. FEBS J., 273: 301-302.

Oz, F., 2006. The effects of different spices on formation of heterocyclic aromatic amines in fresh meat products which cooked at different temperatures. Ph.D. Thesis, Ataturk University Graduate School of Natural and Applied Sciences Department of Food Engineering. Erzurum, Turkey.

Oz, F., G. Kaban and M. Kaya, 2007. Effects of cooking methods on the formation of heterocyclic aromatic amines of two different species of trout. Food Chem., 104: 67-72.

Pais, P., C.P. Salmon, M.G. Knize and J.S. Felton, 1999. Formation of mutagenic/carcinogenic heterocyclic amines in dry-heated model systems, meats and meat drippings. J. Agric. Food Chem., 47: 1098-1108.

Pais, P., M.J. Tanga, C.P. Salmon and M.G. Knize, 2000. Formation of the mutagen IFP in model systems and detection in restaurant meats. J. Agric. Food Chem., 48: 1721-1726.

Skog, K., 2004. Blue cotton, blue rayon and blue chitin in the analysis of heterocyclic aromatic amines: A review. J. Chromatogr. B, 802: 39-44.

Skog, K., K. Augustsson, G. Steineck, M. Stenberg and M. Jägerstad, 1997. Polar and non-polar heterocyclic amines in cooked fish and meat products and their corresponding pan residues. Food Chem. Toxicol., 35: 555-565.

Wakabayashi, K., H. Ushiyama, M. Takahashi, H. Nukaya and S.B. Kim *et al*., 1993. Exposure to heterocyclic amines. Environ. Health Persp., 99: 129-133.

Warzecha, L., B. Janoszka, U. Blaszcyk, M. Stróżyk, D. Bodzek and C. Dobosz, 2004. Determination of heterocyclic aromatic amines (HAs) content in samples of household-prepared meat dishes. J. Chrom. B, 802: 95-106.