THE EFFECT OF MICROWAVE COOKING ON THE FORMATION OF HETEROCYCLIC AROMATIC AMINES IN GOOSE MEAT

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Abstract – In the present study, microwave cooking was evaluated for heterocyclic aromatic amines (HCAs) formation in goose breast and leg meat. 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 IQ was the most commonly found HCA up to 1.44ng/g in the samples, whereas PhIP was not detected in any of the cooked samples. MeAαC was determined only in goose breast meat. The total amounts of HCAs for breast and leg meats were found as 2.20 ng/g and 2.36 ng/g, respectively.

Key Words – Goose Meat, Microwave Cooking, Heterocyclic Aromatic Amines

• INTRODUCTION

Heterocyclic aromatic amines (HCAs) are mutagenic and/or carcinogenic compounds that are formed in meat and fish cooked at temperatures above 150 °C [1]. To date, more than 25 various HCAs have been isolated and identified in foods [2]. 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 [3]. The International Agency for Research on Cancer (IARC) regards some of the HCAs as possible human carcinogens (MeIQ, MeIQx and PhIP, class 2B) and one as a probable human carcinogen (IQ, class 2A) [4].

The concentrations of HCAs depend on meat type, cooking procedures, pH, water activity, carbohydrates, free amino acids, creatin(in)e, heat and mass transfer, lipid, lipid oxidation, antioxidants [5-8]. Human exposure to HCAs is influenced not only by the type of food and cooking method, but also by portion size and intake frequency [9]. To the best of knowledge, the HCA content in goose meat cooked in microwave has not been studied in literature. Therefore, the aim of this study was to investigate the effect of microwave cooking on the formation of HCAs in goose breast and leg meats.

• MATERIALS AND METHODS

Chemicals

HCA standards were purchased from Toronto Research Chemicals (Downsview, Ontario, Canada). The stock standard solutions were prepared according to Öz et al. [8]. For the solid phase extraction, an Oasis MCX cartridge (3 cm^3/60mg, 30µm) of Waters (Milford, Massachusetts, USA) was used. All other chemicals 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 without HPLC grade were

passed through a 0.45-µm filter (Milex, Massachusetts, USA).

Extraction of Heterocyclic Aromatic Amines

HCAs were extracted and purified from the goose meats by using the method described by Messner and Murkovic [10] which is a modified method originally developed by Gross and Gruter [11]. According to the method: 1 g sample of cooked meat was dissolved in 12 ml of 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 of 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.

Identification and Quantification of Heterocyclic Aromatic Amines

HCAs were identified and quantified by HPLC (Thermo Separation Product Spectra System P1000, Thermo Scientific, USA) with Ultraviolet (UV) 3000 detector and AS 3000 auto sampler. Separation was carried out on a reversed phase analytical column (Semi Micro ODS-80 TS column, 5 µm, 250mm × 2 mm i.d) from Tosoh Bioscience GmbH (Stuttgart, Germany) at 30 °C 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 at flow rate 0.3 ml/min. The gradient program was: 0% B, 0-12 min; 0-30% B, 12-20 min; 30% B, 20-25 min. The UV detection of HCAs was performed at 262 nm and the injection volume was 5µl.

- RESULTS AND DISCUSSION

Table 1 LOD, LOQ and recovery values of HCAs

HCAs	LOD (ng/g)	LOQ (ng/g)	Recovery (%)
IQx	0.003	0.01	78
IQ	0.002	0.01	65
MeIQx	0.006	0.02	76
MeIQ	0.005	0.02	79
7,8-DiMeIQx	0.003	0.01	73
4,8-DiMeIQx	0.005	0.02	78
PhIP	0.005	0.02	98
AαC	0.008	0.03	62
MeAαC	0.008	0.03	68

The HCA Content of Goose Meat

This is the first study to describe the levels of HCAs in goose meat in the literature. Table 1 shows the LOD, LOQ and recovery values of individual HCAs. Data from the quantitative HPLC analysis of the HCAs in goose breast and leg meats extracted according to the Oasis method, expressed in ng/g cooked goose meat are presented in Table 2.

Table 2 Data from the quantitative HPLC analysis of the HCAs in goose breast and leg meats

Meat	IQ	IQx	MeIQ	MeIQx	4,8-DiMeIQx	7,8-DiMeIQx	PhIP	AαC	MeAαC	Total HCAs
Breast	1.44	0.07	0.09	0.08	0.08	0.09	nd	0.11	0.24	2.20
Leg	1.08	0.08	0.09	0.8	0.09	0.10	nd	0.12	nd	2.36

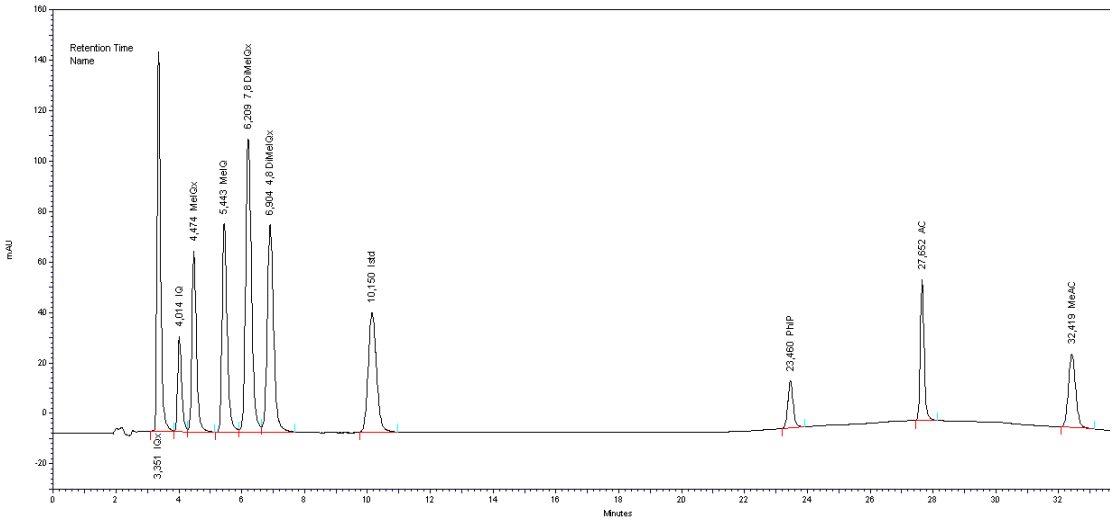
IQ was found as 1.44 ng/g and 1.08 ng/g in breast and leg meats, respectively. IQ was not detected in chicken [1, 3, 6, 12-16], duck [5, 17] and turkey meat [6]. However, Chiu et al. [13]

found that IQ ranged from 0.10 to 0.51 ng/g in fried chicken meat at 200ºC for 5-15min. Turesky et al. [18] also found 0.12 ng/g IQ in barbecued chicken for 20 min. Liao et al. [17] did not detect IQ in roasted, deep-fried and barbecued chicken meat, whereas IQ was found as 1.76 ng/g in pan-fried chicken meat. IQx was also detected at concentrations from 0.07ng/g to 0.08ng/g. IQx was found as 0.07ng/g and 0.08ng/g in breast and leg meats, respectively, whereas IQx was not detected in microwaved [13], fried [6], grilled [15] chicken meat. Liao et al. [17] did not detect IQx in pan fried, deep-fried, roasted and grilled chicken and duck meat, similarly, in turkey meat fried at 275ºC for 30 min [6]. However, Chen and Yang [19] found 0.17ng/g IQx in chicken meat fried at 200°C for 10 min. Chiu et al. [13] also found IQx up to 0.51ng/g in fried chicken meat.

MeIQ was found 0.09 ng/g in both leg and breast goose meats. Similarly, Chen and Yang [19] found 0.11 ng/g in fried chicken at 200°C for 10 min. In other studies, MeIQ was found 1.21 ng/g in fried [13] up to 4.9 ng/g in barbecued [3] chicken meat. MeIQ was also found 0.9 ng/g in fried turkey meat at 140ºC for 20min [20] and up to 2.3 ng/g in roasted turkey meat [3]. In most cooked meat products, MeIQx was reported to occur more frequently than the other HCAs. [1, 3, 6, 17, 18, 20, 21]. In this study, MeIQx was found 0.08ng/g and 0.8ng/g in breast and leg meats, respectively.

4,8-DiMeIQx was found 0.08ng/g and 0.09ng/g in breast and leg meats, respectively. In the literature, 4,8-DiMeIQx was found 0.1ng/g in barbecued chicken [21], 0.5 ng/g in fried chicken at 175-225ºC for 30 min [12], 0,09ng/g in fried chicken at 200ºC for 10 min [19], 0.78ng/g in fried chicken at 200ºC for 5-15min [13], 2.1 ng/g in barbecued chicken for 30 min [3], 0.18 ng/g in fried chicken meat [15]. 7,8-DiMeIQx was found as 0.09ng/g and 0.10ng/g in breast and leg meats, respectively. In other studies, Öz et al. [22] found up to 0.42 ng/g 7,8-DiMeIQx in grilled chicken chops at 200°C for 5-20 min. Up to 1.11 ng/g 7,8-DiMeIQx in pan fried and up to 0.78 ng/g 7,8-DiMeIQx in barbecued chicken chops was also found [22]. In most heat-treated meat, PhIP is one of the most widespread HCAs [3, 6, 14, 16, 17, 18, 23]. However, in this study PhIP was not detected.

Figure 1. HPLC chromatogram of a solution of nine different HCA standards (10ng/g) and internal standard (4,7,8-TriMeIQx, 10ng/g).



AαC was found as 0.11 ng/g and 0.12 ng/g in breast and leg meats, respectively. Similarly, 0.2 ng/g AαC was found in grilled chicken meat (at 175-200ºC for 13min) [14]. In another study AαC was found up to 1.77 ng/g in grilled chicken meat [16]. MeAαC was found 0.24ng/g in breast goose meat. However, MeAαC was not detected in leg goose meat. Chiu et al. [13] found

up to 0.14ng/g MeAαC in microwaved chicken meat for 15 min. On the other hand, the total amounts of HCAs in breast and leg meats were found 2.20ng/g and 2.36ng/g, respectively.

- CONCLUSION

Many previous studies have shown that cooking methods are very important for the formation of heterocyclic aromatic amines. The effect of microwave cooking method on HCA levels in goose breast and leg meat was investigated in the present study. The highest amount of individual HCA detected in the present study belonged to IQ and PhIP was not detected in any of the samples.

REFERENCES

- Knize, M. G., Salmon, C. P., Mehta, S. S. & Felton, J. S. (1997). Analysis of Cooked Muscle Meats for Heterocyclic Aromatic Amine Carcinogens. Mutat. Res. 376: 129–134.
- Sanz Alaejos, M., Ayala, J. H., González, V., & Afonso, A. M. (2008). Analytical methods apply to the determination of heterocyclic aromatic amines in foods. J. of Chrom., B, 862: 15-42.
- Warzecha, L., Janoszka, B., Blaszcyk, U., Stróżyk, M., Bodzek, D. & Dobosz, C. (2004). Determination of Heterocyclic Aromatic Amines (HAs) Content in Samples of Household-Prepared Meat Dishes. J. Chrom. B., 802: 95–106.
- International Agency for Research on Cancer. 1993. IARC monographs on the evaluation of carcinogenic risks to humans. In some naturel occurring substances: food items and constituents Heterocyclic amines and mycotoxins, vol. 56. Lyon: International Agency for Research on Cancer. 163-242.
- Felton, J. S., Malfatti, M. A., Knize, M. G., Salmon, C. P., Hopmans, E. C. & Wu, R. W. (1997). Health Risks of Heterocyclic Amines. Mutat. Res. 376: 37-41.
- Pais, P., Salmon, C. P., Knize, M. G. & Felton, J. S. (1999). Formation of Mutagenic, Carcinogenic Heterocyclic Amines in Dry-Heated Model Systems, Meats and Meat Drippings. J. of Agric. Food Chem., 47: 1098-1108.
- Öz, F. & Kaya, M. (2011). Heterocyclic Aromatic Amines in Meat. J. of Food Proces. & Preserv., 35: 739-753.
- Öz, F., Kaban, G. & Kaya, M. (200). Effects of cooking methods on the formation of heterocyclic aromatic amines of two different species of trout. Food Chem. 104: 67-72.
- Skog, K. (2004). Blue cotton, blue rayon and blue chitin in the analysis of heterocyclic aromatic amines- A review. J. of Chrom. B, 802: 39–44.
- Messner, C. & Murkovic, M. (2004). Evaluation of A New Model System for Studying the Formation of Heterocyclic Amines. J. of Chrom. B. 802 (1): 19–26.
- Gross, G. A. & Grüter, A. (1992). Quantitation of Mutagenic/Carcinogenic Heterocyclic Aromatic Amines in Food Products. J. of Chrom. 592: 271-278.
- Skog, K., Augustsson, K., Steineck, G., Stenberg, M. & Jägerstad, M. (1997). Polar and Non-polar Heterocyclic Amines in Cooked Fish and Meat Products and their Corresponding Pan Residues. Food and Chem. Toxic. 35: 555-565.
- Chiu, C. P., Yang, D. Y. & Chen, B. H. (1998). Formation of Heterocyclic Amines in Cooked Chicken Legs. J. of Food Prot., 61: 712-719.
- Busquets, R., Bordas, M., Toribio, F., Puignou, L. & Galceran, M. T. (2004). Occurrence of Heterocyclic Amines in Several Home-Cooked Meat Dishes of the Spanish Diet. J. of Chrom. B. 802: 79–86.
- Gasperlin, L., Lukan, B., Zlender, B. & Polak, T. (2009). Effects of Skin and Grilling Method on Formation of Heterocyclic Amines in Chicken *Pectoralis Superficialis* Muscle. LWT – Food Sci. Technol. 42: 1313–1319.
- Viegas O., Novo P. and Pinto O. (2012). Ferreira I.M.P.L.V.O., Effect of Charcoal Types and Grilling Conditions on Formation of Heterocyclic Aromatic Amines (HAs) and Polycyclic Aromatic Hydrocarbons (PAHs) in Grilled Muscle Foods, Food and Chem. Toxic. 50(6): 2128-2134
- Liao, G. Z., Wang, G. Y., Xu, X. L. & Zhou, G. H. (2010). Effect of Cooking Methods on

the Formation of Heterocyclic Aromatic Amines in Chicken and Duck Breast. Meat Sci. 85: 149-154.

- Turesky, R. J., Taylor, J., Schnackenberg, L., Freeman, J. P. & Holland, R. D. (2005). Quantitation of Carcinogenic Heterocyclic Aromatic Amines and Detection of Novel Heterocyclic Aromatic Amines in Cooked Meats and Grill Scrapings by HPLC/ESI-MS. J. of Agric. Food Chem. 53: 3248-3258.
- Chen, B. H. & Yang, D. J. (1998). An İmproved Analytical Method for Determination of Heterocyclic Amines in Chicken Legs. J.of Chrom. 48: 223–230.
- Murkovic, M., Friedrich, M. & Pfannhauser, W. (1997). Heterocyclic Aromatic Amines in Fried Poultry Meat. Z. Lebensm. Unters. Forsch. 205: 347–350.
- Murray, S., Lynch, A. M., Knize, M. G. & Gooderham, N. J. (1993). Quantification of the Carcinogens 2-Amino-3,8-dimethyl- and 2-Amino-3,4,8-trimethylimidazo [4,5-f]quinoxaline and 2-Amino-1-methyl-6-phenylimidazo [4,5-b]pyridine in Food using A Combined Assay based on Gas Chromatography-Negative Ion Mass Spectrometry. J. of Chrom. 616: 211–219.
- Öz, F., Kaban, G. & Kaya, M. (2010). Effects of Cooking Methods Levels on Formation of Heterocyclic Aromatic Amines in Chicken and Fish with Oasis Extraction Method. LWT-Food Sci. and Techn. 43: 1345-1350.
- Iwasaki, M., Kataoka, H., Ishihara, J., Takachi, R., Hamada, G. S., Sharma, S., Marchand, L. L. & Tsugane, S. (2010). Heterocyclic Amines Content of Meat and Fish Cooked by Brazilian Methods. J. of Food Com. and Anal. 23: 61-69.