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HETEROCYCLIC AMINES IN FOOD PROCESSING AND ITS FOOD SAFETY ISSUES WTH EMPHASIZING HEALTHY DIET FOR PEOPLE: A MINI REVIEW

Nguyen Thi Minh Tu[1,*], Dang Thuy Linh[1], Vu Thai Hoa[1], Ta Thi Thuy Chi[1], Nguyen Quang Trung[2], Le Tran Ngoan[3,4]

[1] *School of Biotechnolgy and Food Technology Hanoi University of Science and Technology*

[2] *Center for Research and Technology Transfer, Vietnam Academy of Science and Technology*

International Univ. of Health and Welfare, Japan e-mail: letngoan@hmu.edu.vn

[4] *Department of Occupational Health, Hanoi Medical University, Hanoi, Vietnam;*

Abstract

This review introduces heterocyclic amines (HCAs) as a food hazard, its formation during heat assisted process and particularly in food processing is presented. According to International Agency for Research on Cancer, hererocyclic amines have been considered as mutagenic and cancer carcinogenic compounds; and HCAs are evaluated as over 100 fold more mutagenic than aflatoxin B1 and over 2,000-fold more mutagenic than benzo[a] pyrene. Therefore much attention has paid to HCAs recently. Analysis of HCAs also reviewed to emphasize measuring and validation of HCAs in Vietnamese foods for further observational studies in the country.

Key words: Heterocyclic amines, PhIP, MeIQ, MeIQx, food safety, Vietnam.

1. HETEROCYCLIC AMINES

Since the early of XIX century, there were numerous records about the cancer cases of workers working directly in the coalmine. Scientists had then made a hypothesis that the organic compounds under high temperature would result in the compounds potentially leading to cancer. The first published data related to carcinogenic substances (presented in the extracts of roasted foods) were reported in 1939 by E.M.P. Widmark (Widmark, 1939 # 253). He described that female mice treated dermally with an extract from horse muscle that was roasted at high temperature developed mammary tumors. Along with numerous studies showing that cigarette smoke with many carcinogenic compounds, there were a lot heterocyclic amines (HCAs). Then HCAs started to get more and more interests in the field of food safety [1, 2].

Heterocyclic amines are formed from creatine/creatinine, free amino acids and hexoses and they are produced during meat and fish cooking as by-products of the Maillard or browning reaction. The International Agency for Research on Cancer (IARC) has reviewed on cooking methods that produced HCAs [3]. Among them, some of the HCAs are possible for human carcinogens: 2-amino-3,4-dimethyl-imidazo[4,5-f]quinoline (**MeIQ**); 2-amino-3,8-dimethyl-imidazo[4,5-f]quinoxaline (**MeIQx**); 2-amino-1-methyl-6-phenylimidazo [4,5-b] pyridine (**PhIP**); 2-amino-3-methylimidazo [4,5-f] quinoline (**IQ**). The mutagenicity of those HCAs in meat has been assessed by using the microsom test of Ames/Salmonella [4] and it showed that HCAs are over 100 fold more mutagenic than aflatoxin B1 and over 2,000-fold more mutagenic than benzo[a] pyrene [3].

Heterocyclic amines have two major classes: aminoimidazoazoarenes (AIAs) and aminocarbolines. The AIAs are the most important class in cooked foods. They have an imidazo group linked to a quinoline, a quinoxaline or a pyridine. AIAs are also called imidazoquinoline (IQ)-type compounds or thermic HCAs and generated from the reaction of free amino acids creatine, creatinine and hexoses during cooking of foods at conventional cooking temperatures (150-300C) [3].

Now, more than 30 HCAs have been isolated and identified in cooked meat and meat products. Even so, the studies focused on commonly found HCAs in foods such as: IQ (2-Amino-3-methylimidazo[4,5-f]quinoline), **MeIQ**(2-Amino-3,4-dimethylimidazo[4,5-f]quinoline), MeIQx(2-Amino3,8dimethylimidazo[4,5-f]quinoxaline) and PhIP(2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine) [1-3].

2. FORMATION OF HCA IN FOOD PROCESSING

In food processing temperature, processing, cooking time, pH and types of exist amino acid have been reported to affect the formation of HCAs in food [3, 5, 6]. Generally, cooking at higher temperatures and for longer periods of time increase the amount of HCAs produced. Additionally, HCA amount will increase if it uses direct or efficient transfer of heat during cooking. Those food such as fried, grilled, roasted must be considered as HCAs enriched.

PhIP, MeIQ, MeIQx, IQ are formed from the free amino acids, creatine / creatinine and hexose containing in fish, meat. Currently, PhIP, MeIQ, MeIQx, IQ are synthesized in test tubes by heating a mixture of creatine, amino acids such as Phenylalanine, Leucine, Isoleucine, Tyrosine and hexose and they are commercially available for research.

MeIQx and 2-amino-3,4,8 trimethylimidazo[4,5-f]quinoxaline (**DiMeIQx**) were formed in a reaction mixture of threonine, glucose, and creatine at processing temperatures of 150^0C and greater; no heterocyclic amines were detected at lower reaction temperatures. At temperatures of 150^0C, 175^0C, and 200^0C, **MeIQx** and **DiMeIQx** concentrations increased with processing time up to two hours. At higher temperatures of 225^0C or 250^0C, however, **MeIQx** increased during the initial 15 to 30 minutes of processing time, followed by a decrease in concentration, which the authors suggested was due to decomposition.

3. HETEROCYCLIC AMINES AND ITS FOOD SAFETY ISSUES

Human epidemiologic and animal studies have shown that diet plays an important role in cancer development [3]. Impressingly, it has been reported that one third of human cancers are related to foods. Heterocyclic amines are potent mutagens at ng/g levels in cooked foods.

PhIP is one of many HCAs formed when various meats and fish are cooked, with concentrations ranging from 0.56 to 48.5 ng/g in beef and up to 69.2 ng/g in fishes. Originally, PhIP was isolated from fried ground beef cooked at 300^0C and has been detected in cooked beef, chicken, fish, and pork. PhIP is generally found to be the most abundant HCA detected in cooked meats and has been isolated from a complete human diet prepared in a manner to simulate domestic cooking conditions (IARC 1993a) [3]. In the United States where fast food and roast food consumed largely, it is found that, **PhIP** occured at the highest

concentration in roast chicken and at low concentrations in pork. Thus, the consumption value of **PhIP** has been determined by in three major cohorts from the United States (Nurses' Health Study and Health Professionals of the Follow up Study) and the results shoed that this amount is ranged from 285.5 to 457 ng/day[7, 8].

MeIQx has been discovered in meats (beef, pork, chicken and fish) that are usually consumed in the United States. The highest level appears to be in good chicken done grilled and beef (hamburger or steak). **MeIQx** has been detected in some samples of cooked beef, chicken, ground pork and mutton at the concentration up to 30 ng/g (IARC 1993c), while in cooked fishes it can showed up to 72ng/g. Upon heating, MeIQx was formed in concentrated meat juice, and the concentration raised with increased cooking time and temperature. **MeIQ** is present in most foods at much lower concentrations than MeIQx and PhIP, its overall occurrence in food (meat, fish, and broth) has been reported to be less than 1 ng / g. Even that, Augustsson et al. (1999b) reported that about 25% of the population was exposed to measurable amounts of MeIQ in a case-control study from Sweden[9].

4. ANALYSIS OF HETEROCYCLIC AMINES IN FOOD SAFETY CONTROL

PhIP, MeIQ, and **MeIQx** are detected in foods using solid-phase extraction (SPE) and high performance liquid chromatography (HPLC). This method uses diod array and fluorescence detectors to confirm chromatographic peak identities. The SPE-HPLC method allows for detection limits of 1 ng/g PhIP from 10 g of food sample. The liquid chromatography-electrospray-mass spectrometric (LC/ES/MS) method for the determination of **PhIP, MeIQ**, and **MeIQx** has been developed. This method is more sensitive and more stable than the normally used HPLC/ultraviolet (UV) method. LC/ES/MS also allows for simultaneous determination of various HCAs in highly complex matrices such as beef extracts and produces chromatographs that are almost free of interferences. In a complex matrix like beef extracts, the detection limit for PhIP was 0.3 ng/g; for **MeIQ**, 0.3 ng/g; and for **MeIQx**, 1.1 ng/g. A similar method of high-performance liquid chromatography-electrospray tandem mass spectrometry (HPLC/ESI/MS/MS) also has been used for analysis of heterocyclic amines in wines.

MeIQx was identified in food using column chromatography and reverse phase HPLC with analysis by MS and ultraviolet spectrophotometry. The "blue cotton" adsorption technique, in which trisulfocopper phthalocyanine residues are covalently bound to cellulose or cotton, also has been used extensively in

several procedures to detect **MeIQx** in aqueous solutions. One such procedure quantifies **MeIQx** using LC thermospray MS. **MeIQx** is also detected in foods using an SPE and medium-pressure LC method. Replicate samples and spiking allow for accurate determination of extraction losses, and a diode array-ultraviolet detector can confirm chromatographic peak identities. Determination of **MeIQx** levels using these improvements allows for detection limits of 1 ng/g MeIQx from 3 g of meat or 10 g of fish [3]. Gas chromography/MS also has been used to identify MeIQx in fried beef. **MeIQ** was isolated from foods using silica-gel column chromatography and then purifying the sample using reverse-phase HPLC. Later studies used HPLC-thermospray MS to isolate and extract MeIQ from food samples[3].

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References

[1] T. Sugimura, *Nutrition and dietary carcinogens,* Carcinogenesis 21 (2000), 387-395.

[2] T. Sugimura, *Overview of carcinogenic heterocyclic amines,* Mutat Res 376 (1997), 211-219.

[3] IARC, *Some Naturally Occurring Substances: Food Items and Constitutents, Heterocyclic Aromatic Amines and Mycotoxins,* in: WHO-IARC (Ed.), IARC Monographs on the Evaluation of Carcinogenic Risks to humans, WHO-IARC, Lyon France, 1993, pp. 165-229.

[4] M.G. Knize, R. Sinha, N. Rothman, E.D. Brown, C.P. Salmon, O.A. Levander, P.L. Cunningham, J.S. Felton, *Heterocyclic amine content in fast-food meat products,* Food Chem Toxicol 33 (1995), 545-551.

[5] NTP, *Report on Carcinogens,* Thirteenth Edition: Heterocyclic Amines (Selected), http://ntp.niehs.nih.gov/pubhealth/roc/roc13/index.html, U.S. Department of Health and Human Services: National Toxicology Program 2014.

[6] L.C. Maillard, *Action of amino acids on sugars. Formation of melanoidins in a methodical way.,* Compt. Rend. **154** (1912) 66.

[7] N.T. Le, F.A. Michels, M. Song, X. Zhang, A.M. Bernstein, E.L. Giovannucci, C.S. Fuchs, S. Ogino, A.T. Chan, R. Sinha, W.C. Willett, K. Wu, *A Prospective Analysis of Meat Mutagens and Colorectal Cancer in the Nurses' Health Study and Health Professional Follow-up Study,* Environ Health Perspect; http://dx.doi.org/10.1289/EHP238 (2016).

[8] K. Wu, E. Giovannucci, C. Byrne, E.A. Platz, C. Fuchs, W.C. Willett, R. Sinha, *Meat mutagens and risk of distal colon adenoma in a cohort of U.S. men,* Cancer Epidemiol Biomarkers Prev 15 (2006), 1120-1125.

[9] K. Augustsson, K. Skog, M. Jagerstad, P.W. Dickman, G. Steineck, *Dietary heterocyclic amines and cancer of the colon, rectum, bladder, and kidney: a population-based study,* Lancet **353** (1999), 703-707.