**A CARCINOGENE COMPOUND IN MEAT AND MEAT PRODUCTS: HETEROCYCLIC AROMATIC AMINES**

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***ABSTRACT***

*Meat and meat products are of vital importance in human nutrition with their high quality proteins, essential amino acids, vitamins, minerals and high iron content. Raw meat and meat products are not suitable for human consumption due to its “metallic, blood-like, serum-like sweet” and “slightly salty” taste. Thus, they are consumed after cooking by various methods such as grilling, roasting, and frying in oil. With the cooking, many parameters of meat and meat products develop or change such as physical properties (color, size, and tenderness), chemical properties (fat content, protein fraction, increasing flavor and aroma) and microbiological properties (decreasing in microorganism amount). On the other hand, when meat and meat products are exposed to high temperatures for cooking, some heat-induced carcinogenic and mutagenic compounds such as polycyclic aromatic hydrocarbons (PAHs), heterocyclic aromatic amines (HAAs), acrylamide and nitrosamines that adversely affect human health may occur. One of them, HAAs have mutagenic and carcinogenic effects. They are also reported to be one of the causes of many serious health problems for humans such as colon cancer, prostate cancer, colorectal cancer, bladder cancer, breast cancer, rectum cancer, kidney cancer, lung cancer, stomach cancer, pancreas cancer and aerodigestive cancer. Therefore, the formation and reduction strategies of HAAs in meat and meat products are very important to prevent diseases caused by these heat induced contaminants. In this study, it is aimed to make a brief review focusing on the chemical properties of HAAs, their mechanism of formation and precursors, their effects on human health, and methods of reducing HAAs in meat and meat products.*

***KEY WORDS:*** *Meat products, heat treatment, heterocyclic aromatic amines, human health, reducing strategies*

1. **INTRODUCTION**

Heterocyclic aromatic amines (HAAs) are highly carcinogenic and mutagenic compounds that form during the cooking of foods with high protein content at over the 150°C. more than 30 HAAs have been identified in various food products (Alaejos et al., 2008; Haskaraca et al., 2014; Oz and Kotan, 2016). They have been reported as the tumor adjuvant factor in rodents and nonhuman primates (Fu et al., 2014). It is stated that HAAs are 100 times more mutagenic than aflatoxin, and 2000 times more mutagenic than benzo[a]pyrene (Quelhas et al.., 2010). Several studies have revealed the high heat-processed protein based foods can contain high level of these HAAs and they may result in an increased risk for cancer in humans.

Meat produced from beef, lamb, and pork is important source of protein with containing between 18-26% protein, according to meat source (Linseisen et al., 2002; Williams, 2007; Wyness, 2011). With its’ nutritional value and taste it has been consuming since the ancient times, and be one of the primary food of humans. It has been stated that various HAAs are formed during the cooking of these proteinaceous foods. They have been reported as one of the factor that increased the risk of cancer such as colorectal cancer, breast cancer, rectum cancer, kidney cancer, lung cancer, stomach cancer in humans. For this reason, the formation mechanisms of these components, the reduction strategies in foods, and therefore the reduction of cancer risks caused by meat consumption has been one of the research topics that have attracted the attention of researchers for years.

Formation of these heat induced heterocyclic compounds are reported to be affected from cooking type and cooking temperature of meat or presence of precursors and antioxidants in the meat and meat product. Several studies have revealed that the mild or indirect cooking types or lower cooking temperatures can reduce the formation of HAAs. Besides, presence of additives which have antioxidant property or absence of precursors such as sugar, creatine or creatinine can reduce the formation of HAAs. In this study, the formation mechanisms, their effects on human health, and reduction strategies in meat products of heterocyclic aromatic amine formed as a result of heat treatment in meat and meat products are discussed.

1. **CHEMICAL PROPERTIES OF HETEROCYCLIC AROMATIC AMINES**

HAAs are compounds that have a heterocyclic structure. The heterocyclic structure of HAAs consists of 2 to 5 (usually 3) aromatic cycles with 1 or more nitrogen atoms, and usually 1 exocyclic amino group, except for three HAAs which are named as harman, norharman, and 3,4-cyclopentenopyrido [3,2-a] carbazole (Lys-P-1) (Chen and Meng, 1999; Skog, 2002; Bartoszek, 2006; Alaejos and Afonso, 2011; Dong et al., 2020). Heterocyclic amines, which were first detected in 1977 by Sugimura and coworkers in the smoke and burned parts of fish and steaks, show activity in *Salmonella typhimurium*-based mutagenicity tests, and are mutagenic and carcinogenic compounds (Sugimura et al., 1977; Robbana-Barnat et al., 1996; Skog, 2002; Kocadağlı et al., 2017). Today, more than 30 HAAs are detected in various foodstuffs (Nagao et al., 1977; Sugimura et al., 1977; Alaejos et al., 2008; Haskaraca et al., 2014; Yan et al., 2014; Aeenehvand et al., 2016; Karpavičiuté et al., 2017; Yan et al., 2017). The formation of heterocyclic aromatic amines is generally temperature dependent event. Thus, they are divided into two groups according to the formation process and their structure as "thermic HAAs" and "pyrolytic HAAs" (Skog et al., 1998; Alaejos and Afonso, 2011; Kocadağlı et al., 2017; Dong et al., 2020).

Thermic HAAs, which are also known as aminoimidazoazaarenes (AIAs), IQ type, or polar HAAs, are generally formed between 150 °C and 300 °C. Thermic HAAs are also divided into three subgroups as imidazopyridine (IP) type, imidazoquinoline type (IQ), and imidazoquinoxaline type (IQx) according to their chemical structure (Dong et al., 2020; Murkovic, 2004). These amines are produced during Maillard reaction, which is the reaction of free amino acids, creatine/creatinine and hexoses in between 150 °C and 300 °C (Toribio et al., 2002). Pyrolytic HAAs, which are also known as amino carbolines, non-IQ type, or nonpolar HAAs, are commonly formed above 300 °C. Pyrolytic HAAs are also divided into three subgroups as pyridoindole, pyridoimidazole, and other pyrolytic HAAs (Murkovic, 2004; Dong et al., 2020). Pyrolytic HAAs are generated via the pyrolytic reactions of amino acids and proteins (Toribio et al., 2002). Cooking/heating processes that are applied above the 150°C can lead the formation of these carcinogenic and mutagenic HAAS in protein-rich meat and meat products. As meat and meat products have high consumption rates past over the years, knowing of formation and mitigation strategies of these compounds in meat and meat products is very important.

1. **FORMATION MECHANISMS AND THE PRECURSORS OF HAAS**

The formation of HAAs is quite complex. Studies have been carried out with model systems to determine the effects of some physical and chemical parameters on the formation of HAAs and the formation mechanisms of these compounds have been determined (Arvidsson et al., 1997; Pais et al., 1999; Borgen et al., 2001; Solyakov and Skog, 2002; Turesky, 2007; Kataoka et al., 2012). The formation mechanism of thermic HAAs can be explained by the formation mechanism of PhIP, which is the most common HAA in meat and meat products (Felton and Knize, 1991; Dong et al., 2020). Firstly, the phenylacetaldehyde forms from phenylalanine through the Strecker degradation. Later, phenylacetaldehyde reacts with creatinine in aldol reaction to form a mid-product. Lastly, through the subsequent condensation reaction, PhIP forms from this mid-product (Skog et al., 1998; Murkovic et al., 1999; Zöchling and Murkovic, 2002).

As for pyrolytic HAAs, there were several studies about the formation of 𝛽-carbolines including norharman and harman, which occurred in cooked meat, fish, meat extract, and processed meat, such as pizza toppings as salami and cooked ham (Gibis, 2016). Tryptophan was claimed to be the main precursor in the formation mechanism of pyrolytic HAAs, and glucose could facilitate their formation (Pfau and Skog, 2004). Firstly, with the presence of glucose, the tryptophan Amadori rearrangement product is formed from tryptophan. Later, with the presence of reactive carbonyl compounds, it undergoes cyclization (Pictet–Spengler reaction) to form tetrahydro-𝛽-carbolines and thus, 𝛽-carbolines are formed after the oxidation process (Rönner et al., 2000). While norharman and harman can be formed at lower temperatures, the other carbolines are produced at temperatures at which pyrolytic HAAs form (above 300 °C) (Gibis, 2016).

The most common HAAs in cooked meat and fish are given in Table 1. Factors such as cooking time and temperature, cooking type (frying, roasting, grilling), chemical composition of meat such as water activity, pH, carbohydrate, free amino acid, lipid, creatine/creatinine concentration, level of lipid oxidation, and antioxidants are affecting the variety and amounts of HAAs (Jägerstad et al., 1998; Hwang and Ngadi, 2002; Janoszka et al., 2009; Szterk et al., 2012; Pan et al., 2014).

1. **THE EFFECTS OF HAAS ON HUMAN HEALTH**

HAAs, which can produce chromosomal aberrations and sister chromatid exchanges in cultured cells, are mutagenic not only for bacteria, but also for some mammalian cell systems (Alaejos and Afonso, 2011). Therefore, in bacteria and some animals, some of these show 2000 times and 100 times higher mutagenic activity than typical mutagens/carcinogens such as benzopyrene or aflatoxin B1, respectively. There are also HAAs such as harrman and norrharmann that enhance the genotoxicity of mutagenic HAAs (Kawamori et al., 2004; Totsuka et al., 2004; Alaejos et al., 2008; Alaejos and Afonso, 2011). In 1987, six of the known HAAs which are AaC, MeA*a*C, Trp-P-1, Trp-P-2, Glu-P-1 and Glu-P-2 were classified as possible human carcinogens, Group 2B, by the International Agency of Research of Cancer (IARC). Later, in 1993, three of the known HAAs which are MeIQ, MeIQx, and PhIP were classified as possible human carcinogens, Group 2B, by the IARC, and IQ was classified as a probable human carcinogen, Group 2A (IARC, 2020). Furthermore, since the ready-to-eat foods and further processed products are generally prepared with high temperature cooking methods, the amount of HAAs in such foods is significantly high (Rahman et al., 2014).

**Table 1. HAAs in cooked meat, fish and their products** (Jägerstad et al., 1998)

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| **Class** | **Chemical name** | **Abbreviation** |
| **Pyrolytic HAAs** | 2-amino-9H-pirido[2,3-b]indol | A*a*C |
| 2- amino-3-metil-9H-pirido[2,3-b]indol | MeA*a*C |
| 1 metil-9H-pirido[4,3-b]indol | Harman |
| 9H-pirido[4,3-b]indol | Norharman |
| 3-amino-1,4dimetil-5H-pirido[4,3-b]indol | Trp-P-1 |
| 3-amino-1-metil-5H-pirido[4,3-b]indol | Trp-P-2 |
| 2-amino-6-metildipirido[1,2-a;31,21-d]imidazol | Glu-P-1 |
| 2-aminodipirido[1,2-a:31,21-d]imidazaol | Glu-P-2 |
| 2-amino-5-fenilpiridin | Phe-P-1 |
| **Thermic HAAs** | 2-amino-3-metilimidazo[4,5-f]kinolin | IQ |
| 2-amino-3-,4-dimetilimidazo[4,5-f]kinolin | MeIQ |
| 2-amino-3-metilimidazo[4,5-f] kinolin | IQx |
| 2-amino-3,8-dimetilimidazo[4i5-f]kinokzalin | MeIQx |
| 2-amino-3,4,8-trimetilimidazo[4,5-f]kinokzalin | 4,8-DiMeIQx |
| 2-amino-3,7,8-trimetilimidazo[4,5-f]kinokzalin | 7,8-DiMeIQx |
| 2-amino-4-hidroksimetil-3,8-dimetilimidazo[4,5-f] kinokzalin | 4-CH2OH-8-MeIQx |
| 2-amino-1,7,9-trimetilimidazo[4,5-g]kinokzalin | 7,9-DiMeIgQx |
| 2-amino-1,6-dimetil imidazo[4,5-b]piridin | 1,6-DMIP |
| 2-amino-1-metil-6-fenilimidazo[4,5-b]piridin | PhIP |
| 2-amino-1-metil-66-(4-hidroksifenil)-imidazo[4,5-b] piridin | 41-OH-PhIP |
| 2-amino-1,5,6 trimetilimidazo[4,5-b]piridin | 1,5,6-TMIP |
| 2-amino-3,5,6 trimetilimidazo[4,5-b]piridin | 3,5,6-TMIP |
| 2-amino-1,6-dimetilfuro[3,2-e]imidazo[4,5-b]piridin | IFP |

The International Agency for Research on Cancer (IARC) identified consumption of red meat as "probably carcinogenic to humans" (Group 2A) for pancreatic cancer, prostate cancer, especially colorectal cancer, and consumption of processed meat as “carcinogenic to humans” (Group 1) for colorectal cancer as a result of its assessment of the carcinogenicity of red meat and processed meat consumption (IARC, 2015). Since most of the carcinogens cannot have direct mutagenic activity, metabolic enzymes are needed during their activation in the body. Metabolic activation of HAAs begins with N-oxidation by cytochrome enzymes present in the liver and the acetylation reaction takes place. At the end of this reaction, N-acetoxyamine is formed. As N-acetoxyamine is a reactive molecule, it can cause DNA damage and ultimately mutations (Barzegar et al., 2019).

There are many studies on the carcinogenic and mutagenic effects of HAAs. Epidemiological evidence shows that an extreme consumption of well-done red meat containing mutagenic and carcinogenic HAAs may cause certain types of cancer (Zheng et al., 1998; Kampman et al., 1999; Norrish et al., 1999; Stefani et al., 2001; Alaejos et al., 2008). There are many studies in literature conducted to show that heterocyclic aromatic amines may cause certain types of cancer such as prostate cancer (John et al., 2011), colorectal cancer (Rohrmann et al., 2009), bladder cancer (Balbi et al., 2001), breast cancer (Stefani et al., 1997), colon cancer, rectum cancer, kidney cancer (Augustsson et al., 1999), lung cancer (Seow et al., 2000), stomach cancer (Stefani et al., 2001), pancreas cancer (Anderson et al., 2002), and aerodigestive cancer (Stefani et al., 1998).

Rohrmann et al. (2009) reported that if PhIP intake, the most abundant HAAs in the diet, exceeds 41.4 ng/day, it increases the risk of colorectal adenoma compared to an intake of less than 6.5 ng/day. John et al. (2011) indicated that the risk of prostate cancer due to the intake of HAAs was higher among humans who consume hamburgers, processed meat, grilled red meat and well-done red meat. In addition, they found that white meat consumption was not associated with prostate cancer. Seow et al. (2000) found that exposure to heterocyclic amines in fumes emitted during meat cooking may increase the risk of lung cancer among smokers in Chinese women. Research findings show that HAA intake is associated with certain types of cancer in humans, but in some, there is evidence that these types of cancer are not only associated with HAA intake but also with other carcinogenic compounds such as polycyclic aromatic hydrocarbons, N-nitroso compounds, lipid peroxides and other pro-oxidant compounds which are available in well-done meat (Barrett et al., 2003; Cross and Sinha, 2004; Takashi Sugimura et al., 2004).

1. **REDUCING METHODS FOR HAA IN MEAT AND MEAT PRODUCTS**

In recent years, the number of studies on determining the factors affecting the formation of HAAs and reducing or preventing the formation level has increased. There are two main causes of this increase. The first of these is that foods containing HAAs are included in the daily diet of people in many countries. The other is that the cancer rate is high in countries with high consumption of meat and meat products (Felton et al., 1998). In order to reduce HAAs formation in foods there are three main strategies which are adjusting cooking methods or process conditions, adding natural product extracts, antioxidants, or other compounds and properly selecting types of foodstuffs (Dong et al., 2020).

The effect of cooking methods and processing conditions on reducing the formation of HAAs in heat processed meat products has been studied by scientists in detail (Liao et al., 2010; Oz et al., 2010; Guo et al., 2014; Omojola et al., 2015; Oz and Zikirov, 2015; Raza et al., 2015; Oz and Kotan, 2016; Yang et al., 2019). Liao et al. (2010) investigated the effects of various cooking methods such as pan-frying, deep-frying, charcoal grilling and roasting on the formation of HAAs in chicken breast and duck breast. They suggested that the roasting method caused the lowest total HAAs for both chicken and duck breast, followed by deep-frying, pan-frying, and charcoal-grilling methods. Guo et al. (2014) studied the effects of different cooking techniques and time on the formation of HAAs in lamb patties. They concluded that pan-frying generated a higher HAAs content in lamb patties than the frying, roasting and stewing in seasoning, respectively. Also, they added that each cooking method caused an increased formation of HAAs as the cooking time increased. Oz et al. (2010) also studied the relation between doneness and HAAs contents besides the relation between different cooking methods and HAAs contents in chicken and fish. They found that the total HAAs concentrations in chicken and fish products increased with level of doneness for different cooking methods. Oz and Zikirov (2015) found that sous-vide cooking led to lower levels of total HAAs than pan-frying method in beef chops. Moreover, they found that in the sous-vide cooking method, the formation of HAAs increased as the cooking time increased at each temperature.

Many scientists have studied the effects of adding natural product extracts, antioxidants, or other compounds on reducing the formation of HAAs in heat-processed meat products (Quelhas et al., 2010; Gibis and Weiss, 2012; Rounds et al., 2012; Keşkekoğlu and Üren, 2014; Natale et al., 2014; Sabally et al., 2016; Lu et al., 2018; Zeng et al., 2018). Rounds et al. (2012) studied the effects of adding different plant extracts, spices, and essential oils on the formation of HAAs in cooked beef patties. They found that adding olive extract to beef patties led to 79.5% inhibition in the amount of MeIQx and 84.3% in the amount of PhIP; adding apple extract to beef patties led to 76.1% inhibition in the amount of MeIQx and 82.1% in the amount of PhIP; adding clove bud oil to beef patties resulted in 35% inhibition in the amount of MeIQx and 52.1% in the amount of PhIP; adding onion powder to beef patties caused 94.3% inhibition in the amount of MeIQx and 78% in the amount of PhIP; and adding paprika to beef patties led to 68.9% inhibition in the amount of MeIQx and 87.2% in the amount of PhIP. In another study, the effects of rosemary and grape seed extract on the formation of HAAs in fried beef patties were examined by Gibis and Weis (2012). They found that using1.5 g/kg of rosemary extract in sunflower oil reduced the concentration of mix by 57%, and using 8 g/kg of grape seed extract in marinade caused a reduction of about 68% of MeIQx concentration. Also, they found that approximately 90% reduction in PhIP concentration occurred with the use of both extracts at the highest amount. Keşkekoğlu and Üren (2014) investigated the effect of pomegranate seed extract on the formation of HAAs in beef and chicken meatballs cooked in 4 different methods; oven roasting, pan cooking, charcoal-barbecue, and deep-fat frying. They found that pomegranate extract caused a reduction in total HAAs content in charcoal-barbecued and deep-fat fried beef meatballs, by 39% and 46%, respectively. Furthermore, they found that pomegranate extract led to an inhibition of total HAAs content in deep-fat fring chicken meatballs, by 49%. Quelhas et al. (2010) found that green tea extract caused an inhibition of PhIP and AaC in pan-fried beef, by 39% and 46% respectively.

Another way to reduce the formation of HAAs is to choose foodstuff properly, and many studies have been conducted on this subject (Shin et al., 2003; Puangsombat et al., 2012; Zhang et al., 2013; Hasnol et al., 2014; Szterk and Waszkiewicz-Robak, 2014; Wang et al., 2017). Puangsombat et al. (2012) studied the effects of meat types (pork, beef, chicken, and fish) and meat parts on the formation of HAAs (IQx, MeIQx, DiMeIQx, and PhIP). They found that fried pork had higher levels of total HAAs than fried beef and chicken. Wang et al. (2017) found chicken had the lowest amounts of total HAAs in comparison with beef, pork, and mutton. On the other hand, among rock candy, soy sauce and rice wine, soy sauce had the greatest contribution to the formation of HAAs. Szterk and Waszkiewicz-Robak (2014) suggested that the fattening system, animal sex, and refrigerating time of raw meat affected the formation of HAAs. They also found that in rib steak from heifers from a semi-intensive fattening system formed the lowest amount of HAAs during the grilling. Hasnol et al. (2014) studied the effects of different sugar types (table sugar, brown sugar, and honey) on the formation of HAAs in marinated grilled chicken. They suggested that the amounts of all types of HAAs in grilled chicken marinated with table sugar were significantly higher than in chicken marinated with brown sugar and honey. Zhang et al. (2013) found that pork patties had the highest concentration of HAAs compared to pork meatballs and pork strips. They also found that the addition of antioxidant bamboo leaves (the most effective), licorice extract, tea polyphenol, phytic acid, and sodium iso-ascorbate to pork before frying caused an inhibition in the amount of the HAAs content.

1. **CONCLUSION**

As a conclusion, HAAs are carcinogenic compounds formed as a result of heat treatment in meat and meat products. When taken into the body over certain doses, they adversely affect human health and cause many health problems such as colon cancer, prostate cancer, colorectal cancer, bladder cancer, breast cancer, rectum cancer, kidney cancer, lung cancer, stomach cancer, pancreas cancer and aerodigestive cancer. Therefore, it is very important to know the formation and reduction strategies of HAA in meat and meat products in order to prevent diseases caused by these components.

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