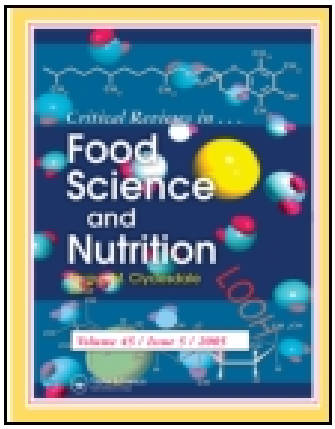

Critical Reviews in Food Science and Nutrition
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/bfsn20

Effect of Thermal Treatment on Meat Proteins with Special Reference to Heterocyclic Aromatic Amines (HAAs)

Muhammad Asim Shabbir[a], Ali Raza[a], Faqir Muhammad Anjum[a], Moazzam Rafiq Khan[a] & Hafiz Ansar Rasul Suleria[a]

[a] National Institute of Food Science and Technology, University of Agriculture, Faisalabad, Pakistan

Accepted author version posted online: 14 Jun 2013.Published online: 08 Aug 2014.

To cite this article: Muhammad Asim Shabbir, Ali Raza, Faqir Muhammad Anjum, Moazzam Rafiq Khan & Hafiz Ansar Rasul Suleria (2015) Effect of Thermal Treatment on Meat Proteins with Special Reference to Heterocyclic Aromatic Amines (HAAs), Critical Reviews in Food Science and Nutrition, 55:1, 82-93, DOI: 10.1080/10408398.2011.647122

To link to this article: http://dx.doi.org/10.1080/10408398.2011.647122

Critical Reviews in Food Science and Nutrition, 55:82–93 (2015)

ISSN: 1040-8398 / 1549-7852 online
DOI: 10.1080/10408398.2011.647122

Taylor & Francis
Taylor & Francis Group

Effect of Thermal Treatment on Meat Proteins with Special Reference to Heterocyclic Aromatic Amines (HAAs)

MUHAMMAD ASIM SHABBIR, ALI RAZA, FAQIR MUHAMMAD ANJUM, MOAZZAM RAFIQ KHAN, and HAFIZ ANSAR RASUL SULERIA

National Institute of Food Science and Technology, University of Agriculture, Faisalabad, Pakistan

Meat is one of the most imperative protein sources available with respect to its production and consumption. It is the richest source of some valuable nutrients like proteins, essential amino acids, polyunsaturated fatty acids, vitamins, and minerals like iron, zinc, and selenium. Thermal treatment produces conformational changes in protein structure as well as flavor, texture, and appearance, and chemical properties of the ingredients are also changed. Heterocyclic aromatic amines (HAAs), potent mutagens/carcinogens, are formed during the cooking of meat at high temperature. The review paper highlights the effects of various cooking methods, i.e., pan-frying, deep-frying, charcoal grilling, and roasting, on the formation of HAAs. The levels of HAAs produced in cooked meats vary depending upon the cooking method, time of cooking, and the type of meat being cooked. Metabolic behavior of HAAs is very unique, they interfere in the activity of many enzymes, modify the metabolic pathways, and lead to the adduct formation of DNA. The application of black pepper and several other spices during processing may reduce the formation of these (HAAs) mutagenic compounds.

Keywords Meat proteins, thermal processing, cooking methods, and heterocyclic aromatic amines

BACKGROUND

Meat is a very important part of our meals, contributing valuable nutrients beneficial to health and accepted by consumers all over the world (Sallam, 2007). It is a primary source of water, fat, proteins, essential amino acids, and some micronutrients like iron, magnesium, selenium, copper, and zinc. Proteins, as a food ingredient, are mostly consumed as an important part of human diet. They provide large amount of essential amino acids required by our bodies. The proteins obtained from animal origin have more nutritional value as compared to those from plants, since the structure and composition of animal meat is similar to that of human (National Health and Medical Research Council, 2006).

Thermal treatment is a physical technique normally used to modify the properties of meat. It involves the use of heat to prepare foods at varying levels of temperature depending upon the type of food and the product being prepared. This application of heat treatment leads to various changes both physical and chemical in the meat component, including protein changes, texture, water-holding capacity, and other important quality factors, such as juiciness, color, and flavor. Morphological changes in muscle tissue during heating have been reported by Palka and Daun (1999). Heat treatment can induce changes in the texture of beef as has been reported previously by Bertola et al. (1994). Effects of thermal processing on the texture of chicken and chicken products (Murphy and Marks, 2000) have been reported. Shrinkage of meat also occurs when fried or roasted, partly due to extrusion and evaporation of water from the meat that decreases meat tenderness and juiciness. These evolutions in meat quality are accompanied by structural changes in the tissue and muscle fibers, depending on heating parameters and muscle composition.

The heating and cooking of foods can result in the formation of large numbers of flavor and aroma constituents. In some cases, however, cooking foods can lead to the formation of chemicals that are detrimental to human health. The Maillard and Browning reactions along with associated side reactions are responsible for the formation of these products. The formation of acrylamide, a carcinogen, in carbohydrate-rich foods, which also contain amino acid precursors, such as asparagine, is an example. Acrylamide has been reported to be formed in quantities exceeding 1 mg/kg in such common foods as French fried potatoes, potato chips, and other food products that are heated to

Address correspondence to Ali Raza, National Institute of Food Science and Technology, University of Agriculture, Faisalabad, Pakistan. E-mail: razi.bwn@gmail.com

high temperatures for preparation. Similar complex chemistries can also lead to other low molecular weight heterocyclic toxic byproducts, such as 3-methylimidazole, and polycyclic aromatic hydrocarbons. Some of these products are favored in the high-temperature cooking of meat and meat products, such as broiling or grilling of meat (Felton and Knize, 1990).

Heterocyclic aromatic amines (HAAs), the potent carcinogens, are formed during the thermal processing of protein-rich foods, especially meat and meat products (Pais et al., 1999). Professor Widmark (1939) was the first person to detect the cancer-causing components in foods. He grilled the horsemeat and than applied its extracts on the back of rats and observed tumor formation. Ames et al. (1975) developed an assay for the measurement of mutagenic activity in bacteria. Carcinogenic compounds were detected in food components, mainly in grilled beef and fish, for the first time by Professor Sugimura and co-workers (Nagao et al., 1977; Sugimura et al., 1977).

MEAT PROTEINS COMPOSITION AND STRUCTURE

Muscles consist of 75% water, 20% protein, 3% fat, and 2% soluble non-protein substances. Out of the latter 2% soluble non-protein substances, metals and vitamins constitute 3%, non-protein nitrogen-containing substances 45%, carbohydrates 34%, and inorganic compounds 18%. Proteins can be divided into three categories, i.e., myofibrillar proteins, sarcoplasmic proteins, and connective tissue proteins. Myofibrillar proteins constitute 50–55% of total protein content. These can be further classified into the following three sub-classes: myofilamentous fibrous proteins, myosin, and actin. The regulatory proteins, including the tropomyosin–troponin complex, α- and β-actinin, M-protein, and C-protein as well as scaffold proteins, such as titin, nebulin, desmin, vimentin, and synemin, also support the myofibrillar structure. The second major myofibrillar protein is actin and the fibrous actin (F-actin) is formed from longitudinal polymerization of globular actin (Ashgar and Pearson, 1980).

Sarcoplasmic proteins account for approximately 30–34% of the total proteins present in muscle fiber. They are soluble in sarcoplasm, including most of the enzymes involved in glycoltic pathway, e.g., creatin kinase, and myoglobin is a major sarcoplasmic protein. The structures built up by connective tissue proteins start with an external covering sheet of connective tissue, the epimysium, around the whole muscle. This layer of connective tissue binds individual bundles of muscle fibers into place, and also binds groups of muscles together. The cell has a membrane, the sarcolemma, and is also surrounded by another type of connective tissue called the endomysium. The fibers are collected into fiber bundles, where the third type of connective tissue (perimysium) envelops the fiber bundle (Ashgar and Pearson, 1980). The third most significant proteins are connective tissue proteins, which comprises about 10–15% of the remaining proteins. This group includes all fibrous proteins: collagen, reticulin, and elastin. Collagen, a glycoprotein, is a main structural component of connective tissues (55–95% of the dry matter content) and is composed of tropocollagen monomers. The tropocollagen molecules aggregate to form either extended fibers in the epimysium and perimysium or mainly as a structural matrix in the endomysium (Knight and Trinick, 1987).

THERMAL PROCESSING

Thermal processing is a core step to prepare meat for cooking purposes, and this makes it digestible. The eating quality of cooked meat depends on the cooking method and the temperature applied which contribute to the development of appearance, flavor, juiciness, tenderness, and other sensory properties as well as chemical properties of the ingredients can also change (Palka and Daun, 1999). During thermal processing a series of biochemical reactions start which alter the structural confirmation of meat proteins. Thermal treatments vary widely across the world, depending upon environmental, economic, and cultural traditions of the region (Plumb et al., 1995). There are many cooking methods that use varying degree of heat and initiate several changes within the muscle fiber. The most widely used methods all over the world are roasting, grilling, deep-frying, and pan-frying (Obuz et al., 2003).

Roasting

In traditional cooking method, meat is cooked in an oven by the steam application. Roasting can also be carried out while revolving meat on a spit over a fire. As it is a dry heat method, a small quantity of fat or oil is used as a base. Temperature reaches up to 200°C during roasting but slow roasting can also be done at 160°C whereas moderate roasting temperature is 170–180°C. Some cuts are best roasted at higher temperature, while others at lower temperature. Beef and lamb cuts due to high marbling are best roasted at low temperature; as a result less shrinkage occurs and serving yields are better. Some mutagenic compounds, known as HAAs, are also formed during roasting. Roasting of chicken and beef burgers in an oven with steam causes low mutagenic activity, as observed by Skog and Jägerstad (1991). This convection of heat reduced temperatures at the surface of products. Felton et al. (1994) suggested that if the meat is kept in microwave for some minutes before frying, the formation of HAAs decreases due to loss of substances in dripping of juices from meat.

Grilling

Grilling is a quick and dry heat method of cooking for tender cuts of meat with radiant heat directed from below or above the meat (Elliott, 1883). Heat transfer to the food when using a grill is primarily via thermal radiation. Direct heat grilling can expose food to temperatures often in excess of 260°C. Grilled meat acquires a distinctive aroma due to a chemical process called the Maillard reaction. Grilling has some health effects as meat is heated over a high temperature and many chemical reactions occur that produce HAAs. Liao et al. (2010) estimated the HAA levels in grilled chicken and duck breasts.

Deep-Frying

In deep-frying, food is immersed in hot oil or fat at a high temperature. This is classified as a dry cooking method because no water is used. The correct frying temperature depends on the thickness and type of the food, but in most cases it lies between 175°C and 190°C. It is a very fast method of cooking certain prime foods that become tasty and texture contracts and crispiness develops. HAAs produced by this frying technique are lower than 1 ng/g.

Pan-Frying

Pan-frying is a fast cooking method for meat in a Teflon-coated pan containing little quantity of oil or fat as its base. Pan should be wide enough so that meat is not crowded during cooking. Too much meat in a small pan reduces temperature and slows cooking. During this method meat is cooked at 180°C for 5–10 minutes. This kind of frying leads to the formation of various kinds of HAAs and their quantities vary from 2 to 70 ng/g of meat cooked. Liao et al. (2010) estimated the levels of various HAAs in pan-fried chicken breast as well as duck.

MEAT PROTEINS INTERACTIONS

Protein denaturation occurs during thermal processing, unfolding of protein starts at 30–32°C, protein–protein association occurs at 36–40°C, gelation occurs at 45–50°C, and finally collagen is denaturated at 53–63°C. The formation of a protein gel subsequent to the unfolding of myofibrillar protein chains has been shown in various studies based upon model systems (Thornberg, 2005).

Sarcoplasmic Proteins

Aggregation of most sarcoplasmic proteins (i.e., muscle proteins soluble in water or at low ionic strength) occurs between 40°C and 60°C, but this could extend up to 90°C. It is also suggested that heat-induced aggregated sarcoplasmic proteins can form a gel within the structural meat constituents by the formation of link between them, which develops consistency in cooked meat (Tornberg et al., 2005). Sarcoplasmic proteins in beef muscle exhibit tenderizing effect in the presence of enzyme at low-temperature, long time heating. It is investigated that collagenase remains active up to 60°C, but when temperature is increased up to 70–80°C, it becomes inactive. Tornberg et al. also showed that a heating time of at least six hours was needed to achieve a substantial lowering of shear force, i.e., a tenderizing effect, and over the same time water loss of 25 to 30% (w/w) had occurred.

Myofibrillar Proteins

The denaturation of myofibrillar proteins in solution usually results in gel formation at a low concentration of 0.5% by weight (Hermansson and Langton, 1988). The gel formation of myosin occurs in two steps at two different temperature ranges. The first part of the reaction occurs between 30°C and 50°C, and the second step occurs above 50°C. The first step involves the aggregation of globular heads of myosin. Sharp and Offer (1992)studied heating of purified myosin at different temperatures for 30 minutes in an electron microscope. They observed that the appearance of myosin molecule did not change after heating at 30°C for 30 minutes. When the temperature was increased up to 35°C, the native myosin molecule was still intact but two myosin molecules started to aggregate by the dimerisation of their heads. Native structure of myosin changed completely at 40°C and only coalesced heads were present, but above 50°C it was too difficult to recognize individual tails and the aggregation was further prolonged. Between 50°C and 60°C large globular aggregates were formed and tails disappeared. In the second stage, helix structure of myosin tail formed network by the interaction of hydrophobic groups.

Connective Tissue Proteins

Differential Scanning Calorimetry (DSC) measurements showed that collagen denaturation occurred between 53°C and

Table 1 Effect of cooking temperature on meat proteins

Degree of doneness	Internal core temperature (±2°C))	Internal description	Approximate oven roasting times @ 160–180°C for primal	Touch test descriptions for grills and pan-fried cuts
Very rare	40–45°C	Internal deep red color, very moist with warm juices, red color	18–20 minutes per 500 g 10–15 minutes resting	Very soft to touch
Rare	45–50°C	Internal very red color, very moist with warmer juices, quite red in color	20–25 minutes per 500 g 10–15 minutes resting	Soft to touch
Medium rare	55–60°C	Internal lighter red color, moist with pink, warm juices	25–30 minutes per 500 g 10–15 minutes resting	Soft and springy to touch
Medium	60–65°C	Internal pink red color, moist with clear pink juices	30–35 minutes per 500 g 10–15 minutes resting	Firm and spongy
Well done	70–75°C	Internal light gray color, a little moist with clear or no pink juices	30–40 minutes per 500 g 10–15 minutes resting	Firm to touch
Very well done	75–80°C	Internal stone gray color, dry with clear or no sign of juices	40–45 minutes per 500 g 10–15 minutes resting	Very firm to touch

63°C. It first involved the breakage of hydrogen bonds loosing up the fibrillar structure, and then the contraction of collagen molecule. If unrestrained, collagen fibers shrink to one-quarter of its resting length on heating to temperatures between 60°C and 70°C. Heat resistant intermolecular bonds are involved in stabilizing of collagen fibers, otherwise they would form gelatin on further heating. So fiber matrix does not dissolve due to the presence of these heat-stable bonds, which retain intermolecular linkages (Light et al., 1985). In young animals the epimysium primarily contains thermally labile cross-links, the perimysium contains a mixture of thermally labile and stable cross-links, and the endomysium contains thermally stable cross-links. As the age of animal increases, the thermally labile cross-links increasingly convert into thermally stable cross-links. Higher levels of heat-stable cross-links lead to the development of greater tension in the connective tissue during cooking (Sims and Bailey, 1981). Structural alterations of proteins occur after heating at 60–80°C for one hour. The collagen present in the epimysuim did not exhibit much alteration after heating. But perimysial as well as endomysial collagen adopt the form of granules at 60°C and their gelatinization is initiated at 80°C. Collagen also shows marked deviation in solublization on heating (Burson and Hunt, 1986).

Protein Aggregation

A Large amount of free radicals are produced during meat cooking, leading to the oxidation of lipids and proteins (Byrne et al., 2002). Basic amino acids, aromatic amino acids, and cysteine are particularly prone to react with free radicals during meat cooking (Santé-Lhoutellier et al., 2008; Gatellier et al., 2009). Most of the time amino acid oxidation is a major cause of protein aggregation. Carbonyle groups are formed due to the oxidation of basic amino acids, which react with free amino acids, and cross-links are induced in proteins, contributing to the aggregation of proteins. Cysteine and tyrosine oxidation can also induce protein cross-links by the formation of intermolecular disulfide and dityrosine bridges, respectively. In addition to these oxidative modifications, the structure of meat is also altered by the implication of thermal treatment, which induces the breaking of hydrogen or electrostatic bonds. As a consequence of this thermal denaturation, an exposure to the protein surface of hydrophobic amino acids can occur that leads to the formation of protein aggregates (Chelh et al., 2006; Santé-Lhoutellier et al., 2008). In cooked meat the relative contribution of thermal denaturation and protein oxidation to the formation of protein aggregates is still unknown. It is considered that protein aggregates are poor substrates for proteases (Grune et al., 2004).

It is studied that protein digestibility decreases due to protein aggregation upon prolonged heating. This reduced digestibility of proteins leads to a decreased bioavailability of amino acids with negative impact on the nutritional quality of meat products. The reduced protein digestibility is not only responsible for poor availability of amino acids but it also has risk factors for human health. As non-hydrolyzed proteins are fermented by colonic flora into mutagenic products, this may cause colon cancer (Evenepoel et al., 1998).

Protein denaturation

Conformational changes occurring in proteins upon the application of thermal treatment are commonly known as denaturation. During this process proteins lose their tertiary structure owing to some external stress, predominately heat treatment. Denatured proteins exhibit a wide range of characteristics from loss of solubility to communal changes. Denaturation of sarcoplasmic and myofibrillar proteins starts at 40–50°C. Further protein denaturation and coagulation takes place at 55°C, leading to myofillment shrinkage and textural toughening. Most of myofibrillar and sarcoplasmic proteins are coagulated at 65°C and the water present in muscles is lost. Shrinkage and denaturation of collagen occur between 60°C and 70°C. The degree of denaturation depends upon the heating time and the temperature applied. The cooking temperature where conformational changes occur is called denaturation temperature and has been mostly investigated using DSC. Other methods generally employed to detect the unfolding of proteins are optical rotary dispersion (ORD) and circular dichroism (CD). Surface hydrophobicity of proteins can also be used to measure the loss of helical structure of proteins, using a fluorescent probe 8-anilino-1-naphtalene sulfonate (ANS). The next step in structural changes to occur on heating is protein–protein interactions, resulting in protein aggregation. These processes are mainly studied by turbidity measurements and loss in protein solubility. The gel-forming ability and the type of gels formed by proteins are usually studied using some kind of mechanical and micro-structural measurements (Christensen, 2000).

PHYSICAL CHANGES IN MEAT PROTEINS

During thermal processing meat undergoes many biochemical changes that affect the appearance, color, flavor, texture, and water-holding capacity. Shrinkage and coagulation of muscle proteins occur upon heating and squeeze out water. The longer the meat is cooked, the more water is forced out. Loss of juices through drip, evaporation, and cooking determines meat's juiciness, the amount of shrinkage, and thus final cooked weight or portion yield. Prolonged cooking or overcooking results in dryness and toughening of meat and it becomes indigestible. Heat affects pigments and changes the color of meat (Young and West, 2001). The red color of uncooked meat changes to light pink and finally to a brown shade, known as degree of doneness. Meat turns lighter upon heating and its brightness increases by increasing temperature and finally turned to brown-gray hue. The lightening is due to an increased reflection of light, arising from light scattering by denatured proteins. The loss of chroma and change in hue result from changes in myoglobin. Myoglobin is

one of the most heat-stable sarcoplasmic proteins, and is almost completely denatured between 80°C and 85°C (Lawrie, 1994). On long, slow cooking some of the connective tissues soften and gelatinize. Slight browning of fat develops flavor, and the more it is browned, the more flavor is developed. Searing or browning the outer lean surface of meat, usually at a fairly high temperature, develops flavor and color through caramelization. It is an important step in several cooking methods, producing tasty meat.

Solubility of Proteins

Changes in the solubility of muscle proteins are obvious during heating. The solubility of denaturated muscle proteins is reduced due to the formation of insoluble aggregates. The contents of soluble proteins, such as sarcoplasmic and myofbrillar, decrease markedly with increasing temperature. Larick and Turner (1992) stated that collagen began to shrink at 60–70°C and was converted to gelatin at 80°C, and that these changes weakened the connective tissue. Similarly Zamri et al. (2006) have reported that muscle hardness increased from 50°C with pH treatment but decreased at 60–70°C. The soluble collagen content increased gradually with increasing temperature from 50 to 100°C. Therefore, reduction in shear value above 80°C could also be due to increased collagen solubility. The collagen solubility decreases with the age of animal because as the animal grows older, the cross-linking between collagen fibers increases and its solubility decreases. For older animals, the more highly cross-linked collagen remained insoluble and shrank during heat treatment (Pearson and Young, 1989).

Texture

The principal proteins responsible for meat texture are stromal and myofibrillar. Texture changes during processing are a result of complex chemical changes. The texture of cooked meat is generally considered to be affected by heat-induced changes in connective tissue, soluble proteins, and myofibrillar proteins. The cross-linkage between collagen molecules within the connective tissue is associated with collagen solubility. Changes in collagen solubility during heating could significantly influence the texture of poultry meat. Heating produces softening of connective tissue caused by conversion of collagen to gelatin and toughening of meat fibers caused by heat coagulation of myofibrillar proteins (Zayas and Naewbanij, 1986).

Water-Holding Capacity

Heat-induced changes in protein solubility also relate to changes in water-holding capacity of meat. Heat-induced modification of myofibrillar protein structure is correlated with water distribution in muscle. Protein's structure changes during thermal processing, and the moisture content held within the narrow channels of myofibrils squeezes out and cook loss occurs. In whole meat, water loss occurs by the shrinkage or swelling of myofibrils and it evaporates from the surface as exudates on cutting of muscle, known as drip loss. Shrinkage of fibers occurs transversely at 40–50°C that enlarges the gap between fibers and the endomysium present around them, whereas the longitudinal shrinkage of muscle fibers and connective tissue network occurs at 60–70°C. As a result of this shrinkage the amount of water held within the tissue system decreases with increasing temperature. This shrinkage causes great water loss that is obtained on cooking. It is then presumed that water is expelled by the pressure exerted by the shrinking connective tissue on the aqueous solution in the extracellular void.

Meat Fiber Shrinkage

Meat fiber shrinkage (MFS) is used to directly measure the shrinkage occurred during cooking of meat. It is based on the investigation carried out on meat shrinkage caused by heat during thermal treatment. MFS is the difference between the raw and cooked areas of the meat sample, expressed as a percentage of the raw area. The method uses a disk of meat (10-mm thick and 55-mm wide) measured before and after cooking in a hot air oven at 165°C for 10 minutes, the meat having reached an internal temperature of 70°C. Video image analysis was used to measure the meat sample area. It can also be used to examine relationship between meat water and shrinkage as well as the effect of meat structure on qualitative analysis (Barbera et al., 2004).

HETEROCYCLIC AROMATIC AMINES (HAAS)

The formation of HAAs in thermally treated meats is based on many factors, including cooking methods, temperature, duration of cooking, and type of meat. High-temperature cooking of meat produces HAAs and their concentration varies in different conditions by more than 100-folds. Amount of HAAs increases by enhancing the temperature and time of cooking (Knize et al., 1994).

The concentration of HAAs produced in cooked meats mostly ranges in parts per billion, especially when cooked by low-temperature traditional cooking methods. Concentrations of HAAs in meats might be increased up to 500 ppb by applying high-temperature treatments like grilling and roasting (Skog et al., 1998). The most commonly found HAAs are 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) and 2-Amino-3,8-dimethylimidazo[4,5-f]quinoxaline (8-MeIQx) formed in grilled poultry, beef, fish, and bacon (Knize et al., 1994). The Maillard reaction has been reported to be involved in the formation of HAAs. It was proposed that amino acids, creatine, and hexoses are the main constituents in meat that act as precursors for the formation these mutagenic compounds (Jägerstad et al., 1983).

Table 2 Chemical names and discovery of heterocyclic aromatic amines (HAAs)

Chemical Names of HAAs	Abbreviation	Year of discovery
3-Amino-1,4-dimethyl-5H-pyrido 4,3-b indole	Trp-P-1	1977
3-Amino-1-methyl-5H-pyrido 4,3-b indole	Trp-P-2	1977
2-Amino-5-phenylpyridine	Phe-P-1	1977
2-Amino-6-methyldipyrido 1,2-a:3,2 -d imidazole	Glu-P-1	1978
2-Aminodipyrido 1,2-a:3,2 -d imidazole	Glu-P-2	1978
2-Amino-9H-pyrido 2,3-b indole	A a C	1978
2-Amino-3-methyl-9H-pyrido 2,3-b indole	MeA a C	1978
2-Amino-3-methylimidazo 4,5-f quinoline	IQ	1980
2-Amino-3,4-dimethylimidazo 4,5-f quinoline	MeIQ	1980
2-Amino-3, 8-dimethylimidazo 4,5-f quinoxaline	MeIQx	1981
4-Amino-6-methyl-1 H-2, 5, 10, 10b-tetraazafluoranthene	Orn-P-1	1981
2-Amino-3,7,8-trimethylimidazo 4,5-f quinoxaline	7,8-DiMeIQx	1984
2-Amino-3,4,8-trimethylimidazo 4,5-f quinoxaline	4,8-DiMeIQx	1985
2-Amino-1-methyl-6-phenylimidazo 4,5-b pyridine	PhIP	1986
2-Amino-3-methylimidazo 4,5-f quinoxaline	IQx	1988
2-amino-1,5,6-trimethyl-imidazopyridine)	TMIP	1988
4-Amino-1,6-dimethyl-2-methylamino-1H,6 H-pyrrolo 3,4-f benzimidazole-5,7-dione	Cre-P-1	1991
2-Amino-1-methyl-6- 4-hydroxyphenyl imidazo 4,5-b pyridine	4 -OH-PhIP	1992
2-Amino-4-hydroxymethyl-3,8-dimethylimidazo 4,5-f quinoxaline	4-CH OH-8-MeIQx	1994
2-amino-3,4,7,8-tetramethylimidazo[4,5-f]quinoxaline	4,7,8-TriMeIQx	1992
2-Amino-1,7,9-trimethylimidazo 4,5-g quinoxaline	7,9-DiMeIgQx	1994

It was reported that creatine contributes to the formation of the amino-imidazol part of the molecule by cyclization and water elimination. Strecker degradation products are responsible for the development of remaining parts of mutagenic imidazoquinolines and imidazoquinoxalines (IQ) compounds. Amino acids and hexoses react with each other through the Maillard reaction and produce pyrazines or pyridines. Aldol condensation joined both the parts together by a Strecker aldehyde. 7,8-DiMeIQx was formed when glucose was heated with glycine, glucose, and creatine at 130°C for two hours (Jägerstad et al., 1984). Threonine was also tested by replacing glycine and resulted in the formation of 4,8-DiMeIQx and MeIQx (Negishi et al., 1985). Alanine was also studied under the same conditions but it formed 4,8-DiMeIQx and trace amounts of MeIQ (Grivas et al., 1985).

Nyhammar (1986) proposed a comprehensive mechanism to produce mutagenic compounds like IQ. It was suggested that condensation reaction occurs between creatine and aldehyde prior to reaction with pyrazine or pyridine. Another hypothesis said that free radical reactions may be involved, e.g., MeIQ and IQ could be the resultant products of reaction between creatinine and free radicals of alkylpyridine (Pearson et al., 1992). Milic et al. (1993) suggested that Strecker reactions along with the Maillard reaction started the production of MeIQx and DiMeIQx, leading to the formation of pyridine- and pyrazine-free radicals and their derivatives with the reaction of creatinine. It was experimentally proved that MeIQx and DiMeIQx were produced when creatinine was heated at 130°C for three hours with 2-methylpyridine, acetaldehyde and 2,5-dimethylpyrazine.

Arvidsson et al. (1997) established an association between temperature and time on the formation of IQ compounds. They heated a mixture of amino acids glucose and creatinine at 150°C and 225°C for 120 minutes. These precursors were in the same ratio as present in animal body. PhIP is an important HAAs and major cause of cancer in humans and other experimental organisms. When creatine and phenylalanine were dry heated together they lead to the formation of PhIP (Felton and Knize, 1990). PhIP could be formed by heating a mixture of tyrosine, leucine, isoleucine, and creatine (Overvik et al., 1989).

Johansson et al. (1995) conducted a study based on liquid model system and described that glucose may be also responsible in promoting the development of PhIP by the reaction of phenylalanine and creatine during heating (Skog and Jägerstad, 1991). It was also studied that if a combined mixture of phenylalanine, creatinine, aldehydes, and sugars is heated, then PhIP could be formed (Manabe et al., 1992). Similar results were obtained by heating a blend of phenylalanine, nucleic acids, and creatinine (Manabe et al., 1993). Experiments based on model systems showed that the production of PhIP from creatinine and phenylalanine was increased exponentially from 0–11 minutes at 150–230°C (Knize et al., 1997). Amazingly, kinetic study suggested that PhIP formation is a mono-molecular reaction in a rate-limiting step (Arvidsson et al., 1997).

Metabolism of HAAs

The metabolic activities of HAAs have been extensively studied in laboratory animals, and experimental trails have been done in human beings under controlled conditions (King et al., 2000). Maximum metabolism of HAAs occurs in the liver of animals, and CYP1A2 is the major cause of oxidation of methyl groups and heterocyclic ring. N-hydroxy-HAA species are produced when exocyclic amine group is bioactivated by CYP1A2-mediated N-oxidation. CYP1A1 and CYP1B1 are also involved in catalyzing the oxidation reaction in tissues outside the liver (King et al., 2000).

DNA adducts are formed in the liver or extra hepatic tissues, where sulfation or acetylation of N-hydroxy-HAAs by SULTs, i.e., sulfotransferases or NATs acetyltransferases, generates extremely weak esters that produce adducts by reacting with DNA. Bioactivation of HAA of N-hydroxy-type substrates is catalyzed by both NAT1 and NAT2, but NAT2 is more active

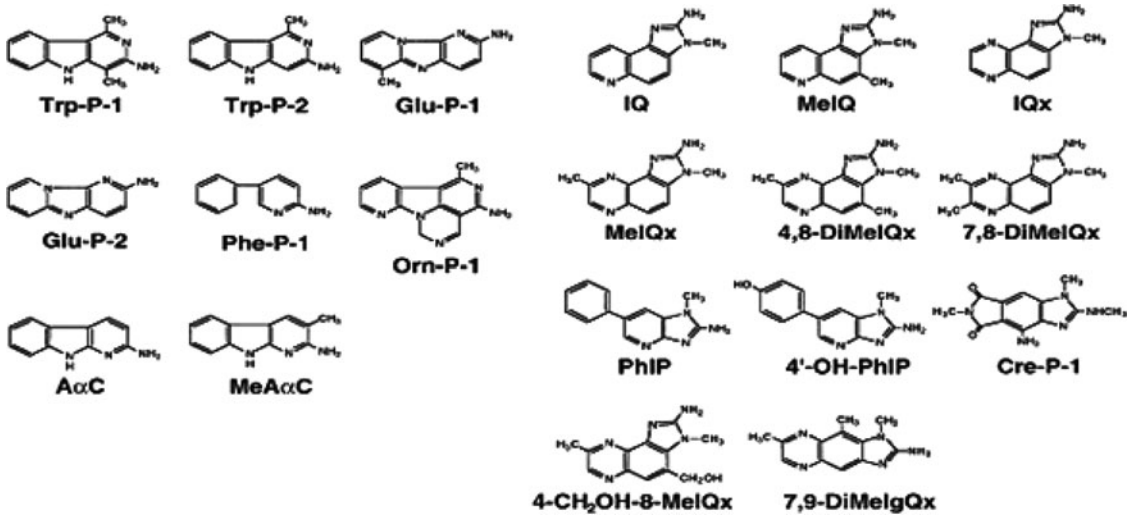

Figure 1 Structures of various heterocyclic aromatic amines (HAAs).

in the catalysis of N-hydroxy metabolites of PhIP and 2-AαC and is preferred for its superiority (King et al., 2000).

HAAs have been bioactivated by several SULTs, e.g., N-hydroxy-PhIP and N-hydroxy-MeAαC are catalyzed by an abundantly used isoform (SULT1A1) (Wu et al., 2000; Glatt et al., 2004). Strong glucuronide conjugates are formed due to detoxication of N-hydroxy-HAAs by UDP-glucuronsyl transferases (UGTs). Different UGTs have been investigated to assess the kinetic parameters of glucuronidation of N-hydroxy-PhIP, but catalysis has been done by active protein isoforms like UGT1A1 (Malfatti and Felton, 2004).

Detoxication of N-hydroxy-HAAs is carried out by NADPH reductases to produce parent amines through reduction back (King et al., 2000). Glutathione S-transferases (GST) is said to be involved in the detoxication of reactive N-acetoxy ester of N-hydroxy-PhIP. It all occurs when conjugation of glutathione (GSH) with its parent amine occurs by reducing a reactive intermediate (Lin et al., 1994). Reductive catalysis mostly occurs by the active enzyme like glutathione S-transferase (GSTA1). UGTs and SULTs are contributed to the formation of detoxicated products of exocyclic amino groups of various HAAs by glucuronidation and sulfamation. Although primary arylamines undergo detoxication through N-acetylation (Hein, 2002), HAAs having N-methyl-aminoimidazole moiety are not possibly detoxicated by this mechanism. This is an unknown mechanism for the detoxication of AαC and many other HAA mutagens even in the presence of NATs (King et al., 2000).

Levels of HAAs in Cooked Foods

Heterocyclic aromatic amines were first reported in 1980s in various protein-rich foods, especially meats heated at high temperatures. At that time the Ames Salmonella assay was mainly used for the estimation of mutagenic activity of HAAs and their quantities. Earlier researchers have many problems in the exact quantification of these carcinogens due to complexity of food mixtures, trace quantities of HAAs, and laborious isolation steps, but recently several new methods have been developed for the extraction, purification, and detection of HAAs (Gross and Gruter, 1992). Data obtained from various food products, especially meat- and fish-based, have suggested that the highest level for PhIP is 480 ng/g. The levels of remaining HAAs are extremely low; even these are not detected in some meat products and mainly range from 50 ng/g for MeIQx to 15 ng/g for 4,8-DiMeIQx, MeIQ, and IQ (Galceran et al., 1996). Crust of cooked meat is the best place for the formation of HAAs and mostly they are detected at this site. But in few investigations trace amounts have been observed in the inner portions of fried meat (Skog et al., 1995).

Fay et al. (1997) reported that HAAs are present in meat and meat products. Meat extracts contain up to 5.9 ng/g HAAs. The amount of IQ in grilled beef is 1.5 ng/g and 0.1 ng/g in 7,8-DiMeIQx. Fumes originated while cooking of meat also contain HAAs. Fumes of frying meat also contain several HAAs. Fried meat's fumes are observed to have about 13.7 pg of MeIQx and 7.3 pg of 7,8-DiMeIQx (Vainiotalo et al., 1993). Smoke emerged from frying meat contained PhIP, MeIQx, and 7,8-DiMeIQx, and their levels were about 6% (Thiebaud et al., 1995).

Genotoxicity of β-carbolines

β-carbolines is a group of HAAs containing 9H-pyrido[3,4-b-]indole (norharman) and 1-methyl-9H-pyrido[3,4-b]indole Harman. Chemically their structural configuration is similar to the breakdown product of amino acids and proteins through pyrolysis, and these mostly occur in meat and fish products heated at high temperature. At the time of their discovery it was believed that amino acid pyrolysates had mutagenic effects, but when purification process of Trp-P-1 and Trp-2 was carried out and non-mutagenic H and NH substances were eliminated, bacterial

Table 3 Effect of cooking methods on the formation of polar heterocyclic aromatic amines (HAAs)

Food type	Cooking method	Temperature	Time (minutes)	IQ	MeLQ	MeLQx	4,8-DiMeLQx	PhIP	Reference
Chicken	Stewed	100		nd		nd	nd	nd	Sinha et al., 1995
Chicken	Broiled			nd	nd	2.33	0.81	38.1	Wakabayashi et al., 1993
Chicken breast	Broiled	180	43	nd	nd	nd	nd	150	Sinha et al., 1995
Chicken	Boiled	100	240			nd	nd	nd	Solyakov and Skog, 2002
Legs with skin	Microwaved	650 w	5–15	nd	nd	nd	nd	nd	Chiu et al., 1998
Bacon	Microwaved	600 w	3			0.1			Gross et al., 1993
Legs without skin	Microwaved	600 w	5–15	nd	nd	nd	nd	nd	Chiu et al., 1998
Chicken legs	Deep-fried	100–200	5–15	0.09	0.51	0.08–0.6	0.78	2.8	Chiu et al., 1998
Beef	Deep-fried	277	7			16.4	4.5	67.5	Thiebaud et al., 1995
Lamb chops	Deep-fried	150–225	9	nd	nd	0.08–0.6	0.04–0.3	2.3	Skog et al., 1997
Meatballs	Deep-fried	150–225	6.5–9	nd	nd	0.8	0.3	0.1	Skog et al., 1995
Salmon	Deep-fried	150	9	0.6	1.3	0.6	0.2	3	Johansson and Jägerstad, 1994
Turkey breast	Pan fried	140	20	1.1	0.9	1.4	0.4	3.8	Murkovic et al., 1997
Chicken	Pan fried	200	10	0.1	0.17	0.13	0.09	0.21	Chen and Yang, 1998
Beef	Pan fried	200–250	6	1		5.1	0.1–0.2	13.3	Felton et al., 1994
Bacon	Pan fried	150–225	4	nd	nd	23.7	1.4	4.5	Skog et al., 1995
Chicken	Roasted	Commercial		nd	nd	2.2	1.3	2.4	Richling et al., 1998
Chicken breast	Roasted	175–240	25–40			1.7	0.3	3	Solyakov and Skog, 2002
Chicken	Grilled	220	40		0.11	0.1		1.4	Tikkanen et al., 1996
Chicken	Grilled	350	20	nd	0.94	0.67	3.4		Knize et al., 1996
Chicken	Grilled	Well done	6	5	100			226	Holder et al., 1997
beef	Barbecued	200–500	15		8	4			Rivera et al., 1996
Beef	Barbecued	180–190	10	nd	nd	1	0.2	nd	Johansson and Jägerstad, 1994
Beef	Barbecued			1.6		4.4	2.7	38	Knize et al., 1995
Flounder	Smoked			0.7	0.3	1.2	0.6	nd	Fay et al., 1997
Salmon	Smoked			0.3	nd	1.3	nd	nd	Johansson and Jägerstad, 1994

nd = not determined.

mutagenicity of tryptophan pyrolysate decreased to a greater level. Carcinogencity was again observed in samples with externally added minute fractions of NH and H (Nagao et al., 1977). Some isomeric forms of H and NH, such as harmine, harmaline, harmol, and harmalol, exist in the form of their alkaloids. These substances are very important due to their biological and pharmacological values.

Kleinbauer and Rabache (1990) studied that NH/H ratio is identical in various pyrolysates of proteins or tryptophan. Many factors are said to be involved in the formation of β-carbolines rather than tryptophan level in proteins. Fried meat also contains H and NH and their amounts range from 8.7–19 ppb and 3–4.8 ppb, respectively (Gross, 1990). Fried fish pyrolysis products contain very high amounts of H and NH, i.e., up to 184 ng/g and 130 ng/g, respectively, and their highest concentrations were found in grilled fish (Gross and Gruter, 1992). Process flavors formation in meat started at about 100–140°C, as at these temperatures H, NH, and other HAAs were not detected (Gross and Gruter, 1992). They observed higher amounts of NH and H in another study. Their levels were 5 ppb for H and 30 ppb for NH (Gross et al., 1993).

Human body was also observed to contain H and NH. Blood platelets contain H, and it was proposed that acetaldehyde and tryptamine form their compounds by condensation. Some alcoholic beverages like whisky, wine beer, brandy, and Japanese saké were reported to contain minor amounts of NH (8.5 ng/mL) and H (0.1 ng/mL) (Adachi et al., 1991). Human urine was also studied to trace out these compounds and were found to range from 97.7–259 ng/g for H and 9.3–33.5 ng/g for NH (Ushiyama et al., 1991). Another evidence for the presence of β-carbolines in human body was obtained from the detection of 1-Methyl-1,2,3,4-tetrahydro-beta-carboline-3-carboxylic acid (MTCA) and 1,2,3,4-tetrahydro-beta-carboline-3-carboxylic acid (TCCA) in milk and urine of normal individuals (Adachi et al., 1991).

Genotoxic Properties

The Ames test was used to testify the mutagenic properties of HAAs, especially Harman and Norharman (Sugimura et al., 1988). Both of these compounds were found to be involved in the modification of metabolic pathways as well as in combining DNA with β-carbolines skeletal structure, known as metabolic potentiation. These HAAs are also responsible for interfering in the activity of different enzymes and many metabolic reactions (de Meester and Vervaet, 1988).

Totsukaa et al. (1999) reported that Harman and Norharman are not real mutagens but act as co-mutagens, and are involved in promoting cancer formation. These β-carbolines (H and NH) could be found in cigarette smoke condensates as well as in different cooked foods. Totsukaa et al. conducted their study on six different types of cigarette brands mostly consumed in Japan. Smoke condensate originated from these

Table 4 Effect of cooking methods on the formation of non-polar heterocyclic aromatic amines (HAAs)

Food type	Cooking method	Temperature (°C)	Time min.	Trp-P-1	Trp-P-2	AαC	MeAα C	Harman	NorHarman	References
Chicken breast	Boiled	100	23					0.1	nd	Solyakov and Skog (2002)
Chicken	Broiled			0.12	0.18	0.21	nd			Wakabayashi et al. (1993)
Chicken breast	Broiled	180	43							Sinha et al. (1995)
Chicken	Boiled	100	240					0.2	0.4	Solyakov and Skog (2002)
Legs with skin	Microwaved	650 w	5–15	0.10–	nd		nd		nd	Chiu et al. (1998)
Bacon	Microwaved	600 w	3							Gross et al. (1993)
Legs, without skin	Microwaved	600 w	5–15	11	nd	0.10	nd	nd	nd	Chiu et al. (1998)
Nuggets	Deep-fried	Commercial		1.03	0.18				0.91*	Richling et al. (1998)
Chicken legs	Deep-fried	100–200	5–15	0.09	0.49	0.23		2.11	1.31	Chiu et al. (1998)
Chicken legs	Pan-fried	200	10	0.18	0.14	0.13		0.012	0.1	Chen and Yang (1998)
Breast	Pan-fried	190	12	3.7	5.1	nd	6.8	nd	nd	Brockstedt and Pfau (1998)
Turkey brest	Pan-fried	190	12	nd	5.1	18.7	nd	12	16.5	Brockstedt and Pfau (1998)
Chicken	Roasted	Commercial								Richling et al. (1998)
Chicken breast	Roasted	175–240	25–40					3.3	1.7	Solyakov and Skog (2002)
Breast clay pot	Roasted	200	25					0.1	nd	Solyakov and Skog (2002)
Breast roasting bag	Roasted	200	25					0.1	0.1	Solyakov and Skog (2002)
Chicken Breast	Grilled	350	20				170			Knize et al. (1996)
Chicken breast	Grilled	Well done	6				100			Holder et al. (1997)
Slices	Grilled	Gas flame	3					133	622	Holder et al. (1997)
Chicken legs	Grilled	Fast food						1.5	1.5	Solyakov and Skog (2002)
Chicken bullion	Stock cubes	100	240					0.5	0.4	Solyakov and Skog (2002)

nd = not determined. *All the values given under Trp-P-1, Trp-P-2, AαC, MeAαC, Harman and NorHarman including asterisk represent the unit value of ng/g

cigarettes along with cooked foods was analyzed by high performance liquid chromatography (HPLC) combined with blue cotton treatment. H and NH were found in smoke condensate of cigarettes. The smoke condensate was divided into two types: mainstream and side stream. Mainstream smoke contains H in the range of 360–2240 ng and NH in the range of 900–4240 ng per cigarette. Side stream contains NH and H in the range of 4130–8990 ng/cigarette and 2100–3000 ng/cigarette respectively. All cooked foods samples were analyzed to measure the contents of these β-carbolines. Cooked foods contain a wide range of H (2.39–795 ng) and NH (0.62–377 ng). So a conclusion can be drawn that exposure of human body to H and NH has increased to much higher levels than HAAs.

Mutagenic chemicals can be divided into the following five classes based on mutational reaction between H and NH: Chemical compounds that have no mutagenic activity; chemical compounds that exhibit potential to cause carcinogenicity in the presence of NH; chemicals that normally show low mutagenicity but can be increased by NH application; mutagenic compounds whose carcinogenic potential is effectively increased by NH; and chemicals which are independent of NH for their mutagenic activity. This last class of compounds loses their mutagenicity in the presence of NH and becomes non-mutagenic.

The data obtained about the co-mutagenicity of NH and H and their derivatives are so variable. They can aggravate mutation by reducing the repairing of DNA damage (Shimoi et al., 1992), enhance mutagens' activity by intercalating in the DNA chain, and produce qualitative differences by affecting distribution between different stages in the growth curve of the test bacteria leading (Sasaki et al., 1992).

H and NH produce carcinogenic metabolite in the presence of amines of aromatic nature. New mutagenic substances are formed from NH by reacting with 3-aminopyridine, an intermediate metabolite. H and NH show weak mutagenic activities toward cultured Chinese hamster cells (Sato et al., 1991). In vitro chemical reaction takes place between DNA and their chemical compounds. The affinity of H is more than that of NH (Nakayasu et al., 1983). The activities of H and NH decreased to a great extent in the presence of their isomeric derivatives such as structurally similar derivatives like Harmaline, Harmanol, and H-armanlol (Ochiai et al., 1986).

Mutagenic characteristics of H and NH in vivo are not studied so much, so a little is known about these in animal bodies. No carcinogenic effect on different organs of mice, especially urinary bladder, has been studied by feeding aniline and NH provided either individually or in mixture (Hagiwara et al., 1980). Yamashita et al. (1988) observed DNA adducts formation in the kidney and liver of mice when fed on a diet containing Harman. DNA adducts were also reported in the stomach and large intestine of mice when NH was added, but these adducts were completely absent in the brain and liver.

Reduction of HAAs

Several experiments have been conducted to reduce the amount of mutagens produced during cooking of food. Miller and Buchanan (1983) observed low mutagenic activity of these HAAs in bacon cooked by low temperature cooking techniques or by decreased levels of heating time. Taylor et al. (1986) removed precursors in the formation of HAAs from beef patties prior to frying by treating them with microwave, and the results obtained by the Ames Salmonella test showed that

mutagenic activity was much lower than without the microwave treatment.

Felton and Knize (1990) devised a new technique to minimize the content of HAAs produced during the frying of ground beef. The mutagenic activity was determined by Salmonella strain TA98 and the quantification of HAAs was done by solid-phase extraction and HPLC. Pretreatment with microwave was carried out at 200°C or 250°C for 0, 1, 2, and 3 minutes before frying. Mutagenic activity was found to be reduced by up to 95% by this treatment as the precursors in the formation of HAAs, such as creatine, creatinine, amino acids, and glucose, were decreased. The final estimation showed that the HAAs present in the fried beef patties were IQ, MeIQx, DiMeIQx, and PhIP and their levels decreased by more than 3–9 folds than the control group.

Hagiwara et al. (1980) carried out a study on fried meatballs to investigate the effect of black pepper on the formation of HAAs. The frying was done at 175, 200, and 225°C. After frying these samples were analyzed and five HAAs were detected. Samples having no black pepper contain 37.81 ng/g of total HAAs, of which 31.80 ng/g were PhIP, while the samples having black pepper before frying contained less HAAs and their amounts decreased by up to 12–100%. PhIP was totally absent and others were also reduced to a greater extent.

CONCLUSIONS

The meat protein composition and structure is correlated with the changes in texture, microstructure, and protein solubility of meat cooked at different temperatures. The behavior of different meat proteins on heating is different. Most of the sarcoplasmic proteins aggregate between 40°C and 60°C, but for some of them the coagulation can extend up to 90°C. For myofibrillar proteins in solution, unfolding starts at 30–32°C, followed by protein–protein association at 36–40°C and subsequent gelation at 45–50°C. The collagen denaturation occurs between 53°C and 63°C, followed by collagen fiber shrinkage. If the collagen fibers are not stabilized by heat-resistant intermolecular bonds, they dissolve and form gelatine on further heating. Structural changes in whole meat and comminuted meat products on cooking result in alterations in water holding, texture, and cooking loss, but collagen solubility increased with increasing internal temperature. In contrast, the solubility of muscle protein decreased with increasing temperature. HAAs, potent mutagens/carcinogens, are formed during the cooking of meat. HAAs are severe mutagens and may increase the risk of certain cancers. More than 20 different HAAs have been identified in cooked foods. HAAs are formed in meat during heating by the interaction of four different substances present in meat, i.e., free amino acids, creatine, creatinine, and sugars. So it can be concluded that the thermally processed meat and meat products showed carcinogenicity and the HAAs were formed as a result of this processing. The contents of HAAs vary in different products on the basis of cooking method and cooking conditions. Some techniques like pretreatment with microwave before cooking of meat and application of certain spices may reduce the formation of these mutagenic compounds, but more study is necessary in this regard, and awareness has to be created among people for this.

REFERENCES

Adachi, J., Mizoi, Y., Naito, T., Yamamoto, K., Fujiwara, S. and Ninomiya, I. (1991). Determination of β-carbolines in foodstuffs by high-performance liquid chromatography and high-performance liquid chromatography mass spectrometry. *J. Chromatogr.* **538**:331–339.

Ames, B. N., McCann J. and Yamasaki, E. (1975). Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsomal mutagenicity test. *Mut. Res.* **31**:347–364.

Arvidsson, P., Boekel, J. S., Skog, K. and Jägerstad, M. (1997). Kinetics of formation of polar heterocyclicamines in a meat model system. *J. Food Sci.* **62**:911–916.

Ashgar, A. and Pearson, A. M. (1980). Influence of ante- and postmortem treatment upon muscle composition and meat quality. *Adv. Food Res.* **26**:55–77.

Barbera, S., Battaglini, L. M. and Tassone, S. (2004). Meat cooking shrinkage: A new parameter to evaluate meat quality. **In**: Procediings of XXXIX Symposium Internazionale di Zootecnia "Meat Science, and Research," pp. 257–264, 10 June 2004, Roma, Italy.

Bertola, N. C., Bevilacqua, A. E. and Zaritzky, N. E. (1994). Heat treatment effect on texture changes and thermal denaturation of proteins in beef muscle. *J Food Process. Preserv.* **18**:31–46.

Brockstedt, U. and Pfau, W. (1998). Formation of 2-amino–carbolines in panfried poultry and 32P-postlabelling analysis of DNA adducts. *Zeitschrift für Lebensmittel-Untersuchung und –Forschung.* **207**:472–476.

Burson, D. E. and Hunt, M. C. (1986). Heat-induced changes in the proportion of types I and III collagen in bovine longissimus dorsi. *Meat Sci.* **17**:153–160.

Byrne, D. V., Bredie, W. L. P., Mottram, D. S. and Martens, M. (2002). Sensory and chemical investigations on the effect of oven cooking on warmed-over flavour development in chicken meat. *Meat Sci.* **61**:127–139.

Chelh, I., Gatellier, P. and Santé-Lhoutellier, V. (2006). Technical note: A simplified procedure for myofibril hydrophobicity determination. *Meat Sci.* **74**:681–684.

Chen, B. H. and Yang, D. J. (1998). An improved analytical method for determination of heterocyclic amines in chicken legs. *Chromatographia.* **48**(3/4):223–230.

Chiu, C. P., Yang, D. Y. and Chen, B. H. (1998). Formation of heterocyclic amines in cooked chicken legs. *J. Food Prot.* **61**:712–719.

Christensen, L. B. (2000). Drip loss. *Meat Sci.* **63**:469–477.

de Meester, C. and Vervaet, C. (1988). Relationships between chem. structure and antimutagenic activity of β-carboline compounds. **In**: Proceedings XVIIIth Annual Meeting of EEMS, Varna, Bulgaria, 3–8 October, 130, Elsevier, Atlanta, GA.

Denoyelle, C. and Lebihan, E. (2003). Intramuscular variation in beef tenderness. *Meat Sci.* **66**:241–247.

Elliott, R. S. (1883). Notes Taken in Sixty Years. R. P. Studley, St. Louis, MO.

Evenepoel, P., Claus, D., Geypens, B., Maes, B., Hiele, M. and Rutgeerts, P. (1998). Evidence for impaired assimilation and increased colonic fermentation of protein related to gastric acid suppression therapy. *Aliment. Pharmacol. Ther.* **12**:10–11.

Fay, L. B., Ali, S. and Gross, G. (1997). Determination of heterocyclic aromatic amines in food products: Automation of the sample preparation method prior to HPLC and HPLC-MS quantification. *Mut. Res.* **376**:29–35.

Felton, J. S., Fultz, E, Dolbeare, F. A. and Knize, M. G. (1994). Effect of microwave pretreatment on heterocyclic aromatic amine mutagens/carcinogens in fried beef patties. *Food Chem. Toxicol.* **32**:897–903.

Felton, J. S. and Knize, M. G. (1990). Heterocyclic amine mutagens/carcinogens in foods. **In**: Handbook of Experimental Pharmacology, pp. 471–502. Copper, C. S. and Grover, P. L., Eds., Springer-Verlag, Berlin, Germany.

Galceran, M.T., Moyano, E., Puignou, L., and Pai, P. (1996). Determination of heterocyclicamines by pneumatically assisted electrospray liquid chromatography-mass spectrometry. *J. Chromatogr.* **730**:185–194.

Glatt, H., Pabel, U., Meinl, W., Frederiksen, H., Frandsen, H., and Muckel, E. (2004). Bioactivation of the heterocyclic aromatic amine 2-amino-3-methyl-9H-pyrido[2,3-b]indole (MeAalphaC) in recombinant test systems expressing human xenobiotic metabolizing enzymes. *Carcin.* **25**:801–807.

Gatellier, P., Kondjoyan, A., Portanguen, S., Grève, E., Yoon, K. and Santé-Lhoutellier, V. (2009). Determination of aromatic amino acid content in cooked meat by derivative spectrophotometry: Implication in nutritional quality of meat. *Food Chem.* **114**(3):1074–1078.

Grivas, S., Nyhammar, T., Olsson, K. and Jägerstad, M. (1985). Formation of a n4ew mutagenic DiMeIQxcompound in a model system by heating creatinine, alanine and fructose. *Mut. Res.* **151**:177–183.

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

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

Gross, G. A., Turesky, R. J., Fay, L. B., Stillwell, W. G., Skipper, P. L. and Tannenbaum, S. R. (1993). Heterocyclic aromatic amine formation in grilled bacon, beef and fish and in grill scrapings. *Carcinogenesis.* **14**:2313–2318.

Grune, T., Jung, T., Merker, K. and Davies, K. J. A. (2004). Decreased proteolysis caused by protein aggregates, inclusion bodies, plaques, lipofuscin, ceroid, and aggresomes during oxidative stress, aging, and disease. *Inter. J. Biochem. Cell Biol.* **36**:2519–2530.

Hagiwara, A., Arai, M., Hirose, M., Nakanowatari, J. I., Tsuda, H. and Ito, N. (1980). Chronic effect of Norharman in rats treated with aniline. *Toxicol. Lett.* **6**:71–75.

Hein, D. W. (2002). Molecular genetics and function of NAT1 and NAT2: Role in aromatic amine metabolism and Carcin. *Mut. Res.* **2**:65–77.

Hermansson, A. M. and Langton, M. (1988). Filamentous structures of bovine myosin in diluted suspensions and gels. *J Sci. Food Agric.* **42**:355–369.

Holder, C. L., Preece, S. W., Conway, S. C., Pu, Y. M. and Doerge, D.R. (1997). Quantification of heterocyclic amine carcinogens in cooked meats using isotope dilution liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry. *Rapid Comm. Mass Spect.* **11**:1667–1672.

Jägerstad, M., Reutersward, A., Dahlqvist, A., Olsson, K., Grivas, S. and Nyhammar, T. (1983). Creatinine and Maillard reaction products as precursors of mutagenic compounds formed in fried beef. In The Maillard Reaction in Foods and Nutrition, ed. G. Waller and M. Feather. p. 507–519. Washington, DC.

Jägerstad, M., Olsson, K., Grivas, S., Negishi, C., Wakabayashi, K., Tsuda, M., Sato S. and Sugimura, T. (1984). Formation of 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline in a model system by heating creatinine, glycine and glucose. *Mut. Res.*; **126**:239–244.

Jägerstad, M., Reutersward, A. L., Olsson, R., Grivas, S., Nyhammar, T., Olsson, K. and Dahlqvist, A. (1983). Creatin(in)e and Maillard reaction products as precursors of mutagenic compounds: Effects of various amino acids. *Food Chem.* **12**(4):255–264.

Johansson, M., Fredholm, L., Bjerna, I. and Jägerstad, M. (1995). Influence of frying fat on the formation of heterocyclicamines in fried beef burgers and pan residues. *Food Chem. Toxicol.* **33**:993–1004.

Johansson, M. and Jägerstad, M. (1994). Occurrence of mutagenic/carcinogenic heterocyclic amines in meat and fish products, including pan residues, prepared under domestic conditions. *Carcinogenesis.* **15**:1511–1518.

King, R. S., Kadlubar, F. F. and Turesky, R. J. (2000). In vivo metabolism of heterocyclicamines. **In**: Food Borne Carcinogens: Heterocyclic Amines, pp. 90–111. Nagao, M. and Sugimura, T., Eds., John Wiley, Chichester, UK.

Kleinbauer, S. and Rabache, M. (1990). The carboline formation by tryptoohan or protein heat treatment. *Sci. Aliment.* **10**:417–428.

Knight, P. and Trinick, J. (1987). The myosin molecule. **In**: Fibrous Protein Structure (Vol. 247). Squire, J. M. and Vibert, P. J. Eds., Academic Press, London.

Knize, M. G., Dolbeare, F. A., Carroll, K. L., Moore, D. H. and Felton, J. S. (1994). Effects of cooking time and temperature on the heterocyclic amine content of fried beef patties. *Food Chem. Toxicol.* **32**(7):595–603.

Knize, M.G., Sinha, R., Rothman, N., Brown, E.D., Salmon, C.P., Levander, O.A., Cunningham, P.L. and Felton, J.S. (1995). Heterocyclic amine content in fast-food meat product. *Food and Chemical Toxicology* **33**:545–552.

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

Knize, M. G., Sinha, R., Salmon, C. P., Mehta, S. S., Dewhirst, K. P. and Felton, J. S. (1996). Formation of heterocyclic amine mutagens/carcinogens during home and commercial cooking of muscle foods. *J. Muscle Food.* **7**:271–279.

Larick, D. K. and Turner, B. E. (1992). Aseptic processing of bovine particulates: Flavor development/stability and texture. *J Food Sci.* **57**:1046–1050.

Lawrie, R. A. (1994). Meat Science. Oxford England: Pergamon Press.

Liao, G. Z., Wang, G. Y. Xu, X. L. and 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.

Light, N., Champion, A. E., Voyle, C. and Bailey, A. J. (1985). The role of epimysial, perimysial and endomysial collagen in determining texture in six bovine muscles. *Meat Sci.* **13**:137–149.

Lin, D. X., Meyer, D. J., Ketterer, B., Lang, N. P. and Kadlubar, F. F. (1994). Effects of human and rat glutathione-S-transferase on the covalent binding of the N-acetoxy derivatives of heterocyclicamine carcinogens in vitro: A possible mechanism of organ specificity in their carcinogensis. *Can. Res.* **54**:4920–4926.

Malfatti, M. A. and Felton, J. S. (2004). Human UDP-glucuronosyl-transferase 1A1 is the primary enzyme responsible for the N-glucuronidation of N-hydroxy-PhIP in vitro. *Chem. Res. Toxicol.* **17**:1137–1144.

Manabe, S., Kurihara, N., Wada, O., Tohyama, K. and Aramaki, T. (1992). Formation of PhIP in a mixture of creatinine, phenylalanine and sugar or aldehyde by aqueous heating. *Carcinogenesis*. **13**:827–830.

Manabe, S., Suzuki, H., Wada, O. and Keki, A. (1993). Detection of the carcinogen, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) in beer and wine. *Carcinogenesis.*; **14**:889–901.

Milic, B. L., Djilas S. M. and Canadanovic-Brun, J. M. (1993). Synthesis of some heterocyclic amino imiazoazarenes. *Food Chem.* **46**:273–276.

Miller, A. J. and Buchanan, R. L. (1983). Reduction of mutagen formation in cooked nitrite-free bacon by selected cooking treatments. *J. Food. Sci.* **48**:1772–1775.

Murkovic, M., Friedrich, M. and Pfannhauser, W. (1997). Heterocyclic aromatic amines in fried poultry meat. *Zeitschrift für Lebensmittel- Untersuchung und -Forschung A.* **205**:34–350.

Murphy, R. Y. and Marks, B. P. (2000). Effect of meat temperature on properties, texture, and cook loss for ground chicken breast patties. *Poultry Sci.* **79**:99–104.

Nagao, M., Honda, M., Seino, Y., Yahagi T. and Sugimura, T. (1977). Mutagenicities of smoke condensates and the charred surface of fish and meat. *Can. Lett.* **2**:221–226.

Nakayasu, M., Nakasato, F., Sakamoto, H., Terada, M. and Sugimura, T. (1983). Mutagenic activity of norharman and harman in chinese hamster lung cells in assays with diphtheria toxin resistance as a marker. *Can. Lett.* **17**:249–255.

National Health and Medical Research Council. (2006). Nutrient Reference Values for Australia and New Zealand. Department of Health and Ageing, Australian Government, Canberra.

Negishi, C., Wakabayashi, K., Yamaizumi, J., Saito, H., Sato, S. and Jägerstad, M. (1985). Identification of 4,8-DiMeIQx, a new mutagen. *Mut. Res.* **147**:267–268.

Nyhammar, T. (1986). Studies on the Maillard reaction and its role in the formation of food mutagens. PhD thesis, Swedish University of Agricultural Science, Sweden.

Obuz, E., Dikeman, M. E. and Loughin, T. M. (2003). Effects of cooking method, reheating, holding time and holding temperature on beef longissimus lumborum and biceps femoris tenderness. *Meat Sci.* **65**:841–851.

Ochiai, M., Wakabayashi, K., Sugimura, T. and Nagao, M. (1986). Mutagenicities of indole and 30 derivatives after nitrite treatment. *Mut. Res.* **172**:189–197.

Overvik, E., Kleman, M., Berg, I. and Gustafsson, J. A. (1989). Influence of creatine, amino acids and water on the formation of the mutagenic heterocyclic amines found in cooked meat. *Carcinogenesis*. **10**:2293–2301.

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

Palka, K. and Daun, H. (1999). Changes in texture, cooking losses, and myofibrillar structure of bovine m. semitendinosus during heating. *Meat Sci.* **51**:237–243.

Pearson, A. M., Chen, C. and Gray, J. I. (1992). Effects of different antioxidants on formation of meat mutagens during frying of ground beef. **In**: 38th ICoMST, pp. 567–570. Clermont-Ferrad, France.

Pearson, A. M. and Young, R. B. (1989). Muscle and Meat Biochemistry. Academic Press, California.

Plumb, G., Mills, E., Tatton, M., D'Ursel, C., Lambert, N. and Morgan, M. (1995). Effect of thermal and proteolytic processing on glycinin, the 11S globulin of soy (Glycine max): A study utilizingmonoclonal and polyclonal antibodies. *J Agric. Food Chem.* **42**:834–840.

Richling, E., Haring, D., Herderich, M. and Schreier, P. (1998). Determination of heterocyclic aromatic amines (HAA) in commercially available meat products and fish by high-performance liquid chromatography electrospray tandem mass spectrometry (HPLC-ESIMS-MS). *Chromatographia.* **48**:258–262.

Rivera, L., Curto, M. J. C., Pais, P., Galceran, M. T. and Puignou, L. (1996). Solid-phase extraction for the selective isolation of polycyclic aromatic hydrocarbons, azaarenes and heterocyclic amines in charcoal-grilled meat. *J. Chromatogr.* **731**:85–94.

Sallam, K. I. (2007). Prevalence of campylobacter in chicken and chicken by-products retailed in Sapporo area, Hokkaido, Japan. *Food Control.* **18**:1113–1120.

Santé-Lhoutellier, V., Astruc, T., Marinova, P., Grève, E. and Gatellier, P. (2008). Effect of meat cooking on physicochemical state and in vitro digestibility of myofibrillar proteins. *J Agric. Food Chem.* **56**:1488–1494.

Sasaki, Y., Yamada, H., Shimoi, K., Kinae, N., Tomita, I., Matsumura, H., Ohta, T. and Shirasu, Y. (1992). Enhancing effects of heterocyclic amines and /3-carbolines on the induction of chromosome aberrations in cultured mammalian cells. *Mut. Res.* **269**:79–95.

Sato, H., Matsuda, K., Iijama, H., Kawahara, T. and Takahashi, M. (1991). Clastogenicity of norharman in cultured Chinese hamster cells. *Mut. Res.* **252**:105–112.

Sharp, A. and Offer, G. (1992). The mechanism of formation of gels from myosin molecules. *J. Sci. Food Agric.* **58**(1):63–73.

Shimoi, K., Kawabata, H. and Tomita, I. (1992). Enhancing effect of heterocyclic amines and β-carbolines on UV or chemically induced mutagenesis in E. coli. *Mut. Res.* **268**:287–295.

Sims, T. J. and Bailey, A. J. (1981). Connective tissue. **In**: Developments in Meat Science – 2, Lawrie, R., Ed., Applied Science, London.

Sinha, R., Rothman, N., Brown, E. D., Salmon, C. P., Knize, M. G., Swanson, C. A., Rossi, S. C., Mark, S. D., Levander, O. A. and Felton, J. S. (1995). High concentrations of the carcinogen 2-amino-1-methyl-6-phenyl-imidazo[4,5-b]pyridine (PhIP) occur in chicken but aredependent on the cooking. *Canc. Res.*; **55**:4516–4519.

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

Skog, K. and Jägerstad, M. (1991). Effects of glucose on the formation of PhIP in a model system. *Carcinogenesis*. **12**:2297–2300.

Skog, K. I., Johansson, M. A. and Jägerstad, M. I. (1998). Carcinogenic heterocyclicamines in model systems and cooked foods: A review on formation, occurrence and intake. *Food Chem. Toxicol.* **36**:879–896.

Skog, K., Steineck, G., Augustsson, K. and Jägerstad, M. (1995). Effect of cooking temperature on the formation of heterocyclicamines in fried meat products and pan residues. *Carcinogenesis*. **16**:861–867.

Solyakov, A. and Skog, K. (2002). Screening for heterocyclic amines in chicken cooked in various ways. *Food Chem. Toxicol.* **40**:1207–1212.

Sugimura, T., Nagao, M., Kawachi, T., Honda, M, Yahagi, T., Seino, Y., Sato, S., Matsukara, N., Shirai, A., Sawamura, M. and Matsumoto, H. (1977). Mutagens-carcinogens in food, with special reference to highly mutagenic pyrolytic products in broiled foods. **In**: Origins of Human Cancer, pp. 1561–1577. Hiatt, H. H., Watson, J. D. and Winsten, J. A., Eds., Cold Spring Harbour Laboratory, New York, NY.

Sugimura, T., Sato, S. and Wakabayashi, K. (1988). Mutagens/carcinogens in pyrolysates of amino acids and proteins and in cooked foods: Heterocyclic aromatic amines. **In**: Chem. Induction of Cancer, Structural Bases and Biological Mechanisms, pp. 681–710. Woo, Y. T., Lai, D. Y., Arcos, J. C. and Argus, M. F., Eds., Academic Press, New York, NY.

Taylor, R. T., Fultz, E. and Knize, M. (1986). Mutagen formation in a model beef supernatant fraction: Elucidation of the role of water in fried ground beef mutagenicity. *Environ. Mut.* **8**:84–85.

Thiebaud, H. P., Knize, M. G., Kuzmicky, P. A., Hsieh, D. P. and Felton, J. S. (1995). Airborne mutagens produced by frying beef, pork and soy-based food. *Food Chem. Toxicol.* **33**:821–828.

Thornberg, E. (2005). Effect of heat on meat proteins – Implication on structure and quality of meat products. *Meat Sci.* **70**(3):493–508.

Tikkanen, L. M., Latva-Kala, K. J. and Heinio, R. L. (1996). Effect of commercial marinades on the mutagenic activity, sensory quality and amount of heterocyclic amines in chicken grilled under different conditions. *Food Chem. Toxicol.* **34**:725–730.

Tornberg, E., Andersson, K. and Josell, A. (1997). The rheological properties of whole and minced meat during cooking as related to sensory and structural characteristics. **In**: Proceedings of the 1st International Symposium on Food Rheology and Structure, 16–20 March, Zurich, Switzerland.

Totsukaa, Y., Ushiyama, H., Ishihara, J., Sinha, R., Goto, S., Sugimura, T. and Wakabayashi, K. (1999). Quantification of the co-mutagenic beta-carbolines, Norharman and Harman, in cigarette smoke condensates and cooked foods. *Can. Lett.* **143**:139–143.

Ushiyama, H., Wakabayashi, K., Sugimura, T. and Nagao, M. (1991). Detection of harman and norharman in human urine. *Mut. Res.* **253**:384–285.

Vainiotalo, S., Matveinen, K. and Reunanen, A. (1993). GC/MS determination of the mutagenic heterocyclic amines MeIQx and DiMeIQx in cooking fumes. *J. Analy. Chem.* **345**:462–466.

Wakabayashi, K., Ushiyama, H., Takahashi, M., Nukaya, H., Kim, S-B., Hirose, M., Ochiai, M., Sugimura, T. and Nagao, M. (1993). Exposure to heterocyclic amines. *Environ. Health Perspect.* **99**:129–133.

Widmark, E. M. P. (1939). Presence of cancer producing substances in roasted food. *Nature.* **143**:972–984.

Yamashita, K., Ohgaki, H., Wakabayashi, K., Nagao, M. and Sugimura, T. (1988). DNA adducts formed by the comutagens harman and norharman in various tissues of mice. *Can. Lett.* **42**:179–183.

Young, O. A. and West, J. (2001). Meat color. **In**: Meat Science Applied, pp. 39–69. Hui, Y. H., Nip, Wai-Kit, Rogers, R. W. and Young, O. A., Eds., Marcel Dekker, New York, NY.

Zamri, Al, Ledward, D. A. and Frazier, R. A. (2006). Effect of combined heat and high pressure treatments on the texture of chicken breast muscle. *J Agric. Food Chem.* **54**:992–2996.

Zayas, J. F. and Naewbanij, J. O. (1986). The effect of microwave heating on the textural properties of meat and collagen solubilization. *J. Food Process. Preserv.* **10**:203–214.