Comprehensive REVIEWS in Food Science and Food Safety

Factors That Affect the Content of Heterocyclic Aromatic Amines in Foods

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Abstract: This review compiles the contents of heterocyclic aromatic amines (HAAs) in foods and beverages, collected from literature data along the period from 1992 up to 2009. Also, it describes the factors that affect the formation of HAAs in foods, such as the cooking method, including temperature, time, and frequency of turning of meat, during cooking. Other factors depending on the type of food and the recipe followed are pH, amounts of HAA precursors, types of amino acids, presence of certain divalent ions, and content of substances with enhancing or inhibiting effects on the formation of HAAs. In addition, there are other factors, which depend on the type of food, such as muscle tissue and the presence of certain genes, since the RN^- allele in pigs increases the glycogen content of muscle. The dispersion of the bibliographic data is evident, and there are scarce data, even no data, referred to individual HAAs. Considering that the diverging results can be due to several causes, possible recommendations are given in order to prevent the dispersion of the results and to achieve more valuable information, applied to determine the HAAs exposure. Although there are not direct indications that HAAs represent a serious health risk to the population, and common cancers are produced by many factors including xenobiotics, all measures to minimize the formation of HAAs should be foreseen, some of which are indicated.

Introduction

To date, more than 25 heterocyclic aromatic amines (HAAs) have been isolated and identified as potent mutagens in the *Ames/Salmonella* test and have been characterized. Table 1 shows their chemical and abbreviated names and some properties. All of these heterocyclic amines contain from 2 to 5 (generally 3) condensed aromatic cycles with 1 nitrogen atom or more in their ring system and, usually, 1 exocyclic amino group, except in the case of Lys-P-1, harman, and norharman. They are formed during the heating process. This formation mainly depends on temperature and thus HAAs are classified at least in 2 groups due to the formation process. HAAs formed at temperatures between 100 and 300 °C are known as "thermic HAAs," IQ type, or aminoimidazoazarenes, and the others formed at higher temperatures, above 300 °C, are known as "pyrolytic HAAs," or non-IQ type. The thermic HAAs are generated from the reaction of free amino acids, creatin(in)e, and hexoses. The precursor undergoes further dehydration and cyclization to form the observed pyrrole and pyridine derivatives. In the Maillard reaction between hexose and amino acids, the formed heterocyclic pyridines and pyrazines undergo further transformation with participation of Strecker aldehydes and creatin(in)e to produce imidazo-quinoxalines, perhaps through free-radical reactions. However, at temperatures as high as 225 and 250 °C, these compounds seem to degrade or react with other compounds (Jackson and Hargraves 1995; Arvidsson and others 1997). In the case of the non-IQ type, the formation takes place through the pyrolytic reaction between amino acids and proteins. Pyrolysis occurs at temperatures higher than 300 °C and produces many reactive fragments through radical reactions. These fragments are believed to condense to form new heterocyclic structures, and pyrolytic mutagens might be formed via free-radical reactions. The mechanisms of formation of Trp-P-1, Trp-P-2, AαC, and MeAαC are unknown. TriMeIQx is a synthetic substance formed in model systems but not in heated foods (Skog and others 1992b). An isomer of 8-MeIQx was discovered in grilled meat and human urine (Holland and others 2004). This isomer is 7-MeIgQx (Turesky and others 2007). Recently, the compounds IQ[4,5-*b*], 7-MeI*g*Qx, and 3 other linear tricyclic ring HAAs, I*g*Qx, 6,7-DiMeI*g*Qx, and 7,9-DiMeIgQx have been identified and quantified in meats cooked under common household conditions (Turesky and others 2005; Ni and others 2008). Therefore, 6 novel compounds that appear to contain the IQx skeleton have also been detected. Two of them have the same nominal molecular weights as IQx and 8-MeIQx. The other 4 are likely to be isomers of DiMeIQx (Turesky and others 2005). The product ion spectra of 2 putative isomers of DiMeIQx are suggestive of structures that contain the amino-*N*-methylimidazo[4,5-*f*]quinoxaline or amino-*N*-methylimidazo[4,5-*g*]quinoxaline ring structure (Table 1). In addition, there are several plausible structures for this presumed isomer of IQx. The identities of these compounds remain to be elucidated (Ni and others 2008).

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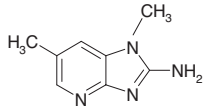
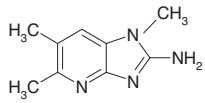
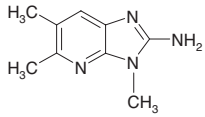
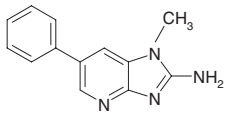
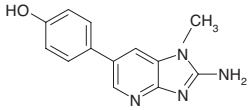
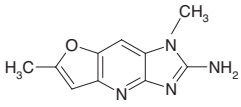
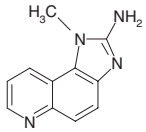
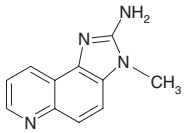
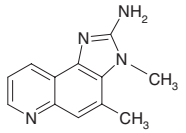
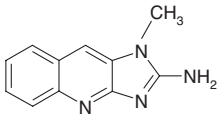
Table 1–Classification of HAAs.

Chemical name	Abbreviated name	Structure	Molecular mass and properties
ISOLATED THERMIC HAAs: AMINOIMIDAZOAZARENES			
Imidazopyridine derivatives			
2-amino-1,6-dimethylimidazo [4,5-b]-pyridine	DMIP		162.2 polar
2-amino-1,5,6-trimethylimidazo[4,5-b]-pyridine	1,5,6-TMIP		176.2 polar
2-amino-3,5,6-trimethylimidazo[4,5-b]-pyridine	3,5,6-TMIP		176.2 polar
2-amino-1-methyl-6-phenylimidazo[4,5-b]-pyridine	PhIP		224.3 pK_a = 5.6 polar
2-amino-1-methyl-6-(4′-hydroxyphenyl)-imidazo[4,5-b]-pyridine	4′-OH-PhIP		240.3 polar
2-amino-1,6-dimethyl-furo[3,2-e]imidazo[4,5-b]-pyridine	IFP		202.3 polar
Imidazoquinoline derivatives			
2-amino-1-methyl-imidazo[4,5-f]-quinoline	iso-IQ		198.2 polar
2-amino-3-methyl-imidazo[4,5-f]-quinoline	IQ		198.2 pK_{a1} = 3,5 pK_{a2} = 6.1 polar
2-amino-3,4-dimethyl-imidazo[4,5-f]-quinoline	MeIQ		212.3 pK_a = 6.4 polar
2-amino-1-methyl-imidazo[4,5-b]-quinoline	IQ[4,5-b]		198.2 polar

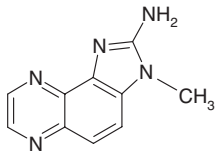
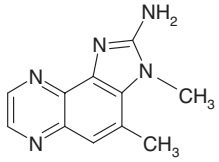
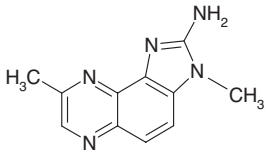
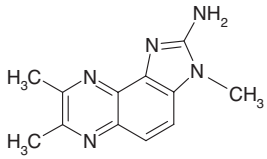
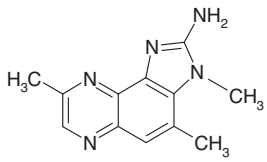
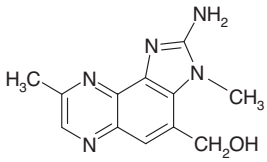
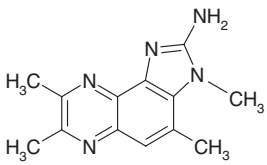
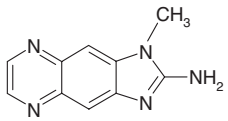
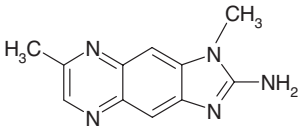
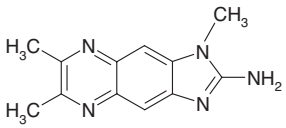
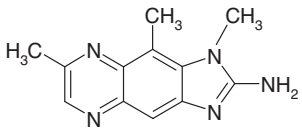
(*Continued*)

According to the chemical behavior of these compounds, they are grouped as polar (aminoimidazoazarenes together with Glu-P-1 and Glu-P-2) and nonpolar (all the others) amines.

The formation of mutagenic compounds (aromatic hydrocarbons) during the cooking of foods, mainly meat and fish products, was reported by Lijinsky and Shubick (1964). Later, Sugimura and others (1977) reported that the mutagenic activity of cooked fish and beef products could not only be justified by aromatic hydrocarbons. This gave as a result the discovery of the HAAs (Hatch and others 1987), which were found to be carcinogenic in long-term animal studies (Bruce 1987).

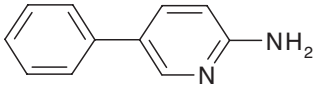
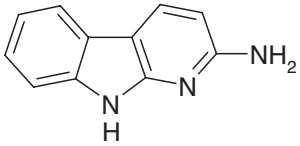
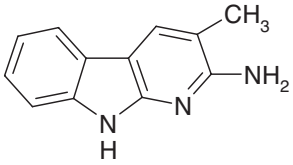
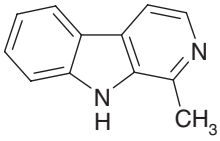
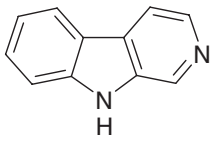
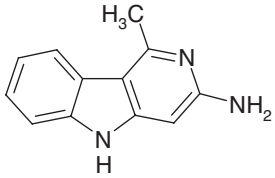
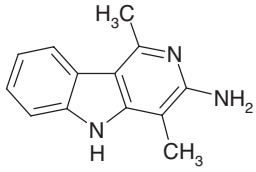
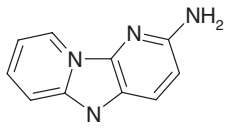
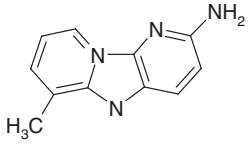
These HAAs are mutagenic not only in bacteria, but also in some mammalian cell systems and can produce chromosomal aberrations and sister chromatid exchanges in cultured cells. Therefore, some of them show much higher mutagenic activity in bacteria and certain animals than typical mutagens/carcinogens such as benzo[a]pyrene or aflatoxin B_1. Harman and norharman do not contain any exocyclic amine group in their structure and they

Table 1–(*Continued*)

Chemical name	Abbreviated name	Structure	Molecular mass and properties
		Imidazoquinoxaline derivatives	
2-amino-3-methyl-imidazo[4,5-f]-quinoxaline	IQx		199.3 polar
2-amino-3,4-dimethyl-imidazo[4,5-f]-quinoxaline	4-MeIQx		213.3 polar
2-amino-3,8-dimethyl-imidazo[4,5-f]-quinoxaline	8-MeIQx		213.3 pK_a = 5.95 polar
2-amino-3,7,8-trimethyl-imidazo[4,5-f]-quinoxaline	7,8-DiMeIQx		227.3 pK_a = 6.5 polar
2-amino-3,4,8-trimethyl-imidazo[4,5-f]-quinoxaline	4,8-DiMeIQx		227.3 pK_a = 5.8 polar
2-amino-4-hydroxymethyl-3,8-dimethyl-imidazo[4,5-f]-quinoxaline	4-CH_2OH-8-MeIQx		243.3 polar
2-amino-3,4,7,8-tetramethyl-imidazo[4,5-f]-quinoxaline	TriMeIQx		241.3 pK_a = 6.0 polar
2-amino-1-methyl-imidazo[4,5-g]-quinoxaline	IgQx		199.3 polar
2-amino-1,7-dimethyl-imidazo[4,5-g]-quinoxaline	7-MeIgQx		213.3 polar
2-amino-1,6,7-trimethyl-imidazo[4,5-g]-quinoxaline	6,7-DiMeIgQx		227.3 polar
2-amino-1,7,9-trimethyl-imidazo[4,5-g]-quinoxaline	7,9-DiMeIgQx		227.3 polar

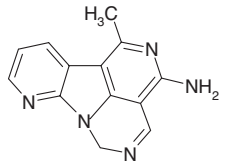
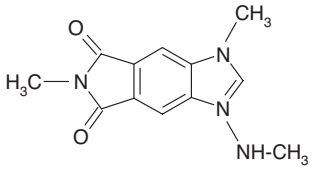
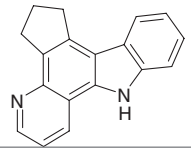
(*Continued*)

Table 1–(*Continued*)

Chemical name	Abbreviated name	Structure	Molecular mass and properties
		ISOLATED PYROLYTIC HAAs: CARBOLINES	
		Phenylpyridine derivatives	
2-amino-5-phenylpyridine	Phe-P-1		170.2 nonpolar
		Pyridoindole derivatives: α-carbolines	
2-amino-9H-pyrido[2,3-b]indole	AαC		183.2 pK_a = 4.4 nonpolar
2-amino-3-methyl-9H-pyrido[2,3-b]indole	MeAαC		197.2 nonpolar
		β-carbolines	
1-methyl-9H-pyrido[3,4-b]indole	Harman		182.3 nonpolar comutagenic
9H-pyrido[3,4-b]indole	Norharman		168.2 pK_a = 6.8 nonpolar comutagenic
		γ-carbolines	
3-amino-1-methyl-5H-pyrido[4,3-b]indole	Trp-P-2		197.4 pK_a = 8.5 nonpolar
3-amino-1,4-dimethyl-5H-pyrido[4,3-b]-indole	Trp-P-1		211.3 pK_a = 8.6 nonpolar
		Pyridoimidazole derivatives δ-carbolines	
2-aminodipyrido-[1,2-a:3′,2′-d]imidazole	Glu-P-2		184.3 pK_a = 5.9 nonpolar
2-amino-6-methyldipyrido-[1,2-a:3′,2′-d]imidazole	Glu-P-1		198.3 pK_a = 6.0 nonpolar

(*Continued*)

Table 1–(*Continued*)

Chemical name	Abbreviated name	Structure	Molecular mass and properties
		Tetraazafluoranthene derivatives	
4-amino-6-methyl-1H-2,5,10,10b-tetraazafluoranthene	Orn-P-1		237.3 nonpolar
		Benzimidazole derivatives	
4-amino-1,6-dimethyl-2-methylamino-1H,6H-pyrrolo-[3,4-f]benzimidazole-5,7-dione	Cre-P-1		244.3 nonpolar
		Carbazole derivatives	
3,4-cyclopenteno-pyrido[3,2-a]carbazole	Lys-P-1		246.3 nonpolar

are not mutagenic, but they enhance the genotoxicity of mutagenic HAAs (Kawamori and others 2004; Totsuka and others 2004). In 1993, the International Agency for Research on Cancer (IARC 1993) considered 8 of the HAAs tested (MeIQ, 8-MeIQx, PhIP, AαC, MeAαC, Trp-P-1, Trp-P-2, and Glu-P-1) as possible human carcinogens (class 2B) and 1 (IQ) as a probable human carcinogen (class 2A) and recommended reducing exposure to these compounds. More recently, in 2004, IQ, MeIQ, 8-MeIQx, and PhIP were listed in the National Toxicology Program as reasonably anticipated to be human carcinogens (NTP Report on Carcinogens 2004). These results are based on the conclusions of long-term animal feeding studies. Although epidemiological evidence suggests that consumption of well done or grilled meat is associated with increased cancer risk in humans, the data are insufficient to support the conclusion that this risk is specifically due to MeIQ, 8-MeIQx, or PhIP present in these foods. Case–control studies show very conflicting results. Nevertheless, it is important to have reliable data on the content of these HAAs in diverse types of foods prepared in different ways in order to assess the effects associated with their intake (NTP Report on Carcinogens 2004).

Many of these HAAs have been isolated from proteinaceous foods including cooked meats and fish, meat extracts, or process flavors. They are also present in several foods (Murray and others 1993; Knize and others 1994b; Thiebaud and others 1995; Tsuchiya and others 1996; Herraiz 2004), coffee (Herraiz 2002; Casal and others 2004; Herraiz 2004), alcohol beverages (Manabe and others 1993c; Rommelspacher and others 1994; Tsuchiya and others 1996; Richling and others 1997), and from environmental sources, such as cooking fumes (Vainiotalo and Matveinen 1993; Vainiotalo and others 1993; Thiebaud and others 1994, 1995), air (Manabe and others 1993b), cigarette smoke (Manabe and others 1993b; Bross and others 1997; Totsuka and others 1999; Sasaki and others 2001; Smith and others 2004), river, and rain water (Wu and others 1995; Ohe 1997; Kataoka and others 2000a; Ono and others 2000). Likewise, some HAAs have been detected in human tissues (Prabhu and others 2001), hair (Reistad and others 1999; Hegstad and others 2000), and in biological fluids, such as plasma, urine, or bile (Friesen and others 1993; Wakabayashi and others 1993; Reistad and others 1997; Kidd and others 1999; Stillwell and others 1999; Strickland and others 2001; Holland and others 2004; Moonen and others 2004; Sentellas and others 2004), as well as in the milk of healthy women (DeBruin and others 2001; Martin and others 2001; Scott and others 2007).

There are some reviews on general aspects concerning HAAs (Wakabayashi and others 1992; Eisenbrand and Tang 1993; RobbanaBarnat and others 1996; Sugimura 1997; Skog and others 1998a; Pais and Knize 2000; Skog 2002), formation in foods (Eisenbrand and Tang 1993; Skog 1993; Chen and Chiu 1998; Jägerstad and others 1998; Skog and others 1998a; Shin and Shin 2003; Murkovic 2004b), in poultry products (Skog and Solyakov 2002; Solyakov and Skog 2002), for carbolines only (DeMeester 1995; Herraiz 2000b; Pfau and Skog 2004), for tryptophan-derived bioactive compounds in food (Herderich and Gutsche 1997), factors determining dietary intakes (Keating and others 1999; Skog 2002), biological significance in the diet (Eisenbrand and Tang 1993; Stavric 1994b; Potter 1996; Norat and Riboli 2001; DeBruin and Josephy 2002; Truswell 2002; Turesky 2002; Berlau and others 2004; Cross and Sinha 2004; Turesky 2004; Jägerstad and Skog 2005; Knize and Felton 2005; Turesky 2007; Sanz Alaejos and others 2008b), genetic polymorphism related to HAAs metabolism (Turesky 2004, 2005; Sanz Alaejos and others 2008c), protective effects (Stavric 1994a; Schwab and others 2000; Vitaglione and Fogliano 2004; Chan and others 2005), biomarkers for exposure assessment (Wild and others 2001; Alexander and others 2002), on sample pretreatment (Hayatsu 1992; Kataoka and others 2000b; Toribio and others 2000a; Skog 2004), and analytical methods applied to their determination (Knize and others 1992; Kataoka 1997; Chen and Chiu 1998; Pais and Knize 2000; Sanz Alaejos and others 2008a). Besides, a recent book (Skog and Alexander 2006) contains a very comprehensive information on these issues. The present article belongs to a series of reviews on

different aspects of HAAs (Sanz Alaejos and others 2008a, 2008b, 2008c), which cover the period from 1992 up to 2009.

Importance of HAAs in human health

The introduction of the Ames test (Ames and others 1975) provided a rapid method of isolating potential carcinogens in food on the basis of their mutagenic activity. But comparing the mutagenic activity in meat samples with the mutagenic activity accounted for by the known HAAs shows that most samples have activity that cannot be explained by the aromatic amines currently identified. This suggests that additional compounds, different from HAAs, are present in these foods and need to be investigated, particularly those grilled over open flames (Balbi and others 2001; Anderson and others 2005; Sinha and others 2005).

Several epidemiological studies have been carried out to test the hypothesis of an association between meat intake and human cancers (Zimmerli and others 2001). In practically all these studies it is difficult to link meat consumption to intake of HAAs. Other substances and factors that possibly contribute to the etiologies of these cancers cannot be excluded, for example, nitrosamines, polycyclic aromatic hydrocarbons (PAHs), acrylamide, high fat or salt intake, physical activity, and others (Wakabayashi and others 1993; Muscat and Wynder 1994; De Stefani and others 1997b, 1998, 2001; Singh and Fraser 1998; Zheng and others 1998; Augustsson and others 1999; Bingham 1999; Sinha and others 1999, 2001; Balbi and others 2001). Nonetheless, epidemiological studies appear to imply that consumption of very well done red meat in excess may induce certain types of cancer (De Stefani and others 1997a; Zheng and others 1998; Bingham 1999). However, other studies have shown no such association (Muscat and Wynder 1994; Augustsson and others 1999; Kampman and others 1999; Skog 2002). Experts from the World Cancer Research Fund (WCR) and the American Inst. for Cancer Research (1997) reviewed data on red meat intake and cancer risk. Those experts concluded that high intake of red meat *probably* increases the risk of developing colorectal cancer and *possibly* increases the risk of pancreas, breast, prostate, and kidney cancers. The influence of cooking methods on the risk of cancer was also evaluated. The report concluded that there is no convincing evidence that any method of cooking modifies the risk of any cancer, neither exists evidence of any probable causal relationship (WCR 1997). The results of studies on cooking methods are not consistent. Nonetheless, to date, there are few data to evaluate the dose response of the possible relationship between HAAs and human cancer. Besides, as diet is a complex mixture that contains carcinogens, cocarcinogens, and anticarcinogens, a simplistic approach of evaluating only HAAs may not be appropriate (Sugimura and others 1996; Sinha and Rothman 1999). The presence of certain genotypes related to HAA metabolism seems to enhance notably any cancer risk development (Sanz Alaejos and others 2008c). Thus, genetic predisposition seems to be the main factor in cancer development related to HAAs and/or other mutagenic compounds.

A major difficulty of the epidemiological studies of intake of HAAs is to estimate the exposure to HAAs (Sinha and Rothman 1997; Keating and others 1999; Keating and Bogen 2001; Alexander and others 2002; Skog 2002). Dietary assessment in combination with analytical data on HAA levels in several foods is used to estimate intakes of predominant HAAs such as PhIP and 8-MeIQx. To obtain such assessments, food frequency questionnaires that include not only type and amount of foods consumed, but also methods of preparation and level of doneness are required. There are several errors on this approach including bias, inconsistent reporting, difficulty in quantifying cooking doneness by such methods, and the day-to-day variation in the diet. Some methods have been developed to estimate dietary HAA levels using HAA concentrations in experimentally cooked meats reported in the literature and meat consumption data obtained from dietary surveys. Possibilities and problems have been analyzed (Sinha and others 1996; Sinha and Rothman 1999; Voskuil and others 1999; Keating and Bogen 2001). For example, a main problem when using literature data on HAAs in cooked dishes is that many experiments have been carried out under unspecified cooking conditions or at high cooking temperatures and for long times to maximize the production. These conditions can be nonrepresentative of the way in which the general population usually cooks meats in a region. The relation between the degree of doneness and surface browning may differ because some people fry their meat at a high temperature for a short time to obtain a brown surface with the interior not cooked through, while others fry their meat at a lower temperature but for a longer time. This can lead to the same degree of surface browning but very different HAA amounts. Cooking practices and eating habits differ among different populations, and conclusions drawn from 1 population may not be applicable to other groups (Sinha and Rothman 1997; Keating and others 1999; Keating and Bogen 2001, 2004; Alexander and others 2002; Skog 2002). In some countries, pan residues are used to prepare gravies (Olsson and others 2005; Busquets and others 2008; Janoszka and others 2009), and pan residues contain substantial amounts of HAAs compared with the corresponding food item. Meat extracts and bouillon (cubes) also contribute to HAA intakes. Therefore, it is important to establish databases on HAA contents in cooked foods that are representative for the eating habits of the population being studied and to take into account each ingredient of the recipe: spices, condiments, and so on. The dishes should be prepared in a way that reflects normal household and restaurant cooking conditions. Sometimes color photos are used to assess the preference of the consumer on the degree of doneness and, indirectly, the amount of HAAs (Sinha and Rothman 1997; Keating and others 1999; Keating and Bogen 2001; Alexander and others 2002; Skog 2002; Olsson and others 2005). The results can lead to an underestimation of exposure (Skog 2002). Also, HAA levels in home-cooked meat samples were significantly different when samples were visually classified for doneness, but not when self-reported for doneness preference (Keating and others 2000). For commercially prepared foods, considerable differences between equivalent products from different manufacturers are found in many cases (Tikkanen and others 1993). This variation indicates that industrial processing of foods has a marked effect on the mutagenic activity of the product (Tikkanen and others 1993). Dietary reports from frequency of consumption questionnaires show 20- to 110-fold relative variation in certain HAAs, when the 10th and 90th percentiles of daily dietary HAA intakes are compared (Byrne and others 1998).

The accurate determination of HAAs is a difficult analytical task, since traces of these compounds have to be determined in highly complex food matrices. This problem can only be solved by combining both elaborate sample preparation steps with selective separation steps followed by detection methods allowing the quantification of HAAs at low levels. Usually, tedious cleanup procedures that include extraction, purification, and preconcentration steps, followed by a separation technique, such as liquid or gas chromatography and capillary electrophoresis are used. The main detection systems used are ultraviolet (UV), fluorescence, electrochemical, and mass spectrometry (Hayatsu 1992; Knize and

others 1992; Kataoka 1997; Chen and Chiu 1998; Kataoka and others 2000b; Pais and Knize 2000; Toribio and others 2000a; Skog 2004, Sanz Alaejos and others 2008a). Furthermore, the results obtained from different analytical methods might not be comparable. Some HAAs or HAA derivatives can bind with other food components. All of these formed compounds cannot be extracted from food by the usual extraction methods and, therefore, the dietary assessment of genotoxic compounds may be underestimated. Therefore, different extraction procedures are applied to cooked and uncooked meat, before and after enzymatic proteolysis (Martin and others 2002). Thus, problems in determining appropriate estimates of extraction recovery rates must also be taken into account.

In order to overcome some of the problems connected with the use of questionnaires in the assessment of exposure to HAAs, attempts have been made to develop biomarkers. Biomarkers of diet promise to provide a more accurate measure of dietary intake. They are more objective because they do not rely on people's memory when being interviewed or on the accuracy of recording the food diary. But, since HAAs do not accumulate in body tissues, the use of biomarkers does not solve the problem of exposure estimation back in time for years. Hence, HAAs in body fluids or tissues would only give a measure of recent exposure (Wild and others 2001).

Mechanisms of formation

The HAA precursors in meat and fish are creatin(in)e, hexoses, free amino acids, and some dipeptides, all common compounds of muscle tissue. Thus, when heating a mixture of creatinine, glucose, carnosine, and a blend of amino acids, in proportions similar to those found in bovine meat, formation is found of the polar HAAs IQx, 8-MeIQx, 4,8-DiMeIQx, 7,8-DiMeIQx, and PhIP and the apolar HAAs harman and norharman (Arvidsson and others 1997, 1999; Pais and others 2000). But 8-MeIQx is formed from 17 different amino acids in a model system, making difficult the assignment of a formation pathway (Johansson and others 1995a). And in model systems, HAAs can be formed with or without sugars. However, in meats, a minimum (or critical) sugar concentration is important for formation of HAAs (Skog 1993). The incorporation of carbon atoms originated from ^{14}C-labeled glucose into the imidazoquinoxaline derivates (IQx, 8-MeIQx, and 4,8-DiMeIQx) has been demonstrated; therefore, glucose is a precursor in the formation of these HAAs (Skog and Jägerstad 1993). Other models were constructed by combining glucose, creatinine, and phenylalanine (Phe) in all the possible ways. The results showed that HAAs could only be formed in both systems of glucose and creatinine, and of Phe, glucose, and creatinine, and the amounts of the HAAs formed were lower in the former system (Chen and Meng 1999). The formation of HAAs and the degradation of these precursors occurred simultaneously (Ahn and Grun 2005a).

Some studies (Sugimura 1997; Jägerstad and others 1998; Murkovic 2004b) have postulated that the products from the Maillard reaction, such as pyridines or pyrazines formed by Strecker degradation, undergo an aldol-type condensation. The resulting vinyl-pyridines or vinyl-pyrazines go through a conjugation with creatinine. The reaction is completed by ring closure, water elimination, and desaturation to give imidazoquinolines, imidazoquinoxalines, and imidazopyridines.

Other studies suggest a direct condensation between an aldehyde and creatinine. The creatinine molecules serve as basis for the aminomethylimidazo ring typical of most HAAs, after cyclization and water elimination. This reaction occurs quickly if the temperature of the meat is higher than 100 °C. The formation of PhIP was studied by heating Phe and creatine (Felton and others 1999). The 3-carbon atom, the amino nitrogen, and the intact phenyl ring from Phe are incorporated into PhIP, as well as the methyl carbon, the 1-nitrogen, and the amino nitrogen from labeled creatine. These results seem to indicate that the carbon atoms of Phe form a part of the pyridine moiety and the creatine forms the imidazol part (Felton and others 1999).

Another mechanism of formation of PhIP has been proposed by using ^{13}C-labeled Phe as a reaction partner in a model system containing additional creatinine (Murkovic and others 1999). Isolation of the labeled reaction product (PhIP) and ^{13}C-nuclear magnetic resonance experiments show that the carbon atoms of Phe form a part of the pyridine moiety. The 6-membered pyridine ring is completed by formation of a Schiff's base and cyclization. The optimal conditions are 2 h for reaction time and 200 °C, because at room temperature no formation of PhIP occurred. Immediate cleanup is necessary since PhIP disappeared completely when the reaction mixture was left overnight at room temperature. It could be due to its binding to melanoidins that are formed during the reaction. PhIP was still found in high amounts when part of the Phe was replaced by phenylacetaldehyde in the model system. Phenylacetaldehyde is a Strecker degradation product of Phe and is a common intermediate of Maillard browning reaction in foods, and in the proposed mechanism phenylacetaldehyde plays a key role that undergoes an aldol condensation with creatinine. Thus, ^{13}C-phenylacetaldehyde itself was formed by pyrolytic degradation of ^{13}C-2-Phe under the experimental conditions (Murkovic and others 1999). In other experiments, ^{15}N-labeled Phe was used (Zöchling and Murkovic 2002). The suggested mechanism is as follows the 1st reaction is the thermal degradation of Phe to ^{15}N- or ^{13}C-phenylacetaldehyde. No ^{13}C-labeled PhIP is found when the carboxyl carbon of Phe is labeled. This suggests that the carbon dioxide resulting from decarboxylation of Phe does not contribute any further to the formation of PhIP. The 2nd step is the aldolization between phenylacetaldehyde and creatinine. The C-5 of creatinine reacts with phenylacetaldehyde in a nucleophilic addition to form 2-amino-1-methyl-5-(1′-hydroxy-2′-phenylethyl)-imidazol-4-one) and subsequent dehydration to form the condensation product as an intermediate. The final step of a possible reaction mechanism could be the formation of a Schiff's base between the creatinine part of the condensation product and a compound with an amino group (Murkovic and others 1999; Zöchling and Murkovic 2002). This last compound can be Phe, 2-phenylethylamine, creatinine, or even ammonia instead of amino acids. In a model reaction with phenylacetaldehyde and creatinine, PhIP was produced without an additional nitrogen source. The identification of the phenylacetaldehyde, the aldol addition, and the aldol condensation products were unequivocal. The formation of the Schiff's base would explain why position 5 is labeled when ^{13}C-2-Phe is used as reactant (Zöchling and Murkovic 2002).

PhIP has also been found in mixtures of creatinine, Phe, and nucleic acids heated at 60 °C for 4 wk. Nucleic acids, deoxy- and ribonucleotides, as well as deoxy-ribose and ribose induce the formation of PhIP in the presence of Phe and creatinine (Manabe and others 1993a).

Some mutagens have a 2-amino-imidazole moiety, and this suggests that arginine could contribute to the guanidino fragment for this group (Knize and others 1994a).

Free-radical intermediates, carbon-centered radicals, and pyridine and pyrazine cation radicals generated in the Maillard reaction are involved in the production of imidazoquinoxaline-type HAAs

(Milic and others 1993; Kikugawa 1999). The initial step in the formation of 8-MeIQx and 4,8-DiMeIQx depends on the existence and kinetics of the Maillard and Strecker reactions and pyridine and pyrazine free-radical formation. Stabilization of the formed free radicals occurs in the 2nd step, giving pyridine and pyrazine derivatives. Later, these compounds react with creatinine to form amino-imidazoazarenes (Milic and others 1993).

The addition of 4 Maillard reaction products enhances the mutagenicity of pork juice after boiling (Lee and others 1995). The highest level of enhancement is observed with tetrahydrothiophene and 2,3-dimethylpyrazine, suggesting that these 2 Maillard reaction products are probably involved in the formation of IQ-type mutagens in boiling pork juice. However, the addition of 2-acetylpyrrole and imidazole greatly inhibits the mutagenicity of pork juice (Lee and others 1995).

The kinetics of formation of some HAAs have been investigated with a meat juice model system, obtained from roasted beef by pressing (Arvidsson and others 1999), and with a model system prepared with precursors, such as creatinine, carnosine, amino acids, and glucose in proportions similar to those found in bovine meat (Arvidsson and others 1997). Kinetics of formation and subsequent degradation, respectively, could reasonably be described by these 1st-order models:

$$C_t = C_0(1 - e^{-k_1 t}) \text{ and } C_t = C_0(e^{k_2 t} - e^{-k_1 t})$$

In these equations, C_t is the concentration of a HAA as a function of time, C_0 the concentration of the compound from which the HAA is formed (both expressed in mmol HAA/mmol creatinine), t the heating time (min), k_1 (min^{-1}) the rate constant for degradation of C_0 and at the same time the rate constant for formation of C_t, and k_2 is the rate constant for the degradation of HAAs (min^{-1}) (Arvidsson and others 1997). The temperature dependence of the rate constants of formation was analyzed using the Eyring equation

$$k = \frac{K_b T}{h} e^{\frac{\Delta S*}{R}} e^{-\frac{\Delta H}{RT}}$$

rather than the Arrhenius equation ($k = A \exp(-E_a/RT)$) because the Eyring equation gave the activation entropy ΔS^*, which may give an indication about the reaction mechanism. k is the rate constant, K_b is the Boltzmann constant, and h is the Planck constant. Negative values indicate a bimolecular reaction, whereas at near zero values a monomolecular reaction. The calculated ΔS^* indicates that the rate-limiting step forming PhIP follows a monomolecular reaction, while for all the IQx derivatives the formation follows a bimolecular reaction of pseudo-1st-order, for example, one of 2 reactants in large excess. Perhaps, the rate-limiting step might be the reaction between creatinine-aldehyde and pyrazine (Arvidsson and others 1997, 1999).

The stability of 15 HAAs standards was kinetically studied during heating at 100, 150, and 200 °C for different periods of time (Chiu and Chen 2000). Results show that the HAAs loss increased both when increasing temperature and heating time, and the degradation rate of each HAA during heating fits a 1st-order model. The formation and degradation processes could proceed simultaneously during heating. The results obtained in this study (Chiu and Chen 2000) are quite similar to those reported by Arvidsson and others (1997). PhIP is also the most susceptible to degradation during heating, and this instability may be attributed to the following reasons: (1) PhIP contains more conjugated carbon–carbon double bounds, which make it more susceptible to chemical change than the other HAAs; (2) PhIP contains a benzene ring attached to a single bond of a side chain, which makes it free to rotate (Chiu and Chen 2000).

β-carbolins can be formed by oxidation of the tetrahydro-β-carbolins (THβC). The so-called Pictet–Spengler reaction of indoleethylamines with aldehydes or α-keto acids has been proved to be the most efficient route of THβC formation. Cooking conditions, water content, temperature, and pH affect the reaction, in which Try and formaldehyde or acetaldehyde are implicated (Totsuka and others 1999; Herraiz 2000b). The Schiff base formed from an indoleamine is cyclized in acidic medium to give THβC (Herraiz 2000b). β- and γ-carbolines were found in model systems after heating at 180 °C during 10 min a mixture of creatinine, glucose, and various single amino acids (Johansson and others 1995a).

Aqueous and dry model systems were used to investigate the effects of water on HAAs formation (Skog and others 2000). When creatinine was added to the closed aqueous model system, the amounts of IQx and 8-MeIQx increased, but the amount of norharman decreased, and PhIP was not detected. In particular, notably fewer amounts of aminoimidazoazarenes were detected in the open dry system. In the dry system, AαC was formed at normal cooking temperatures and the yield of PhIP increased dramatically, in contrast to the IQ-compounds. A possible explanation for the differences in PhIP formation, related to water content, could be in terms of water and free-radical quenching. Water has a considerable influence on the species of HAAs. Dry heating favors the formation of AαC (Skog and others 2000), DMIP is 2-amino-1,6-dimethylimidazo [4,5-b]-pyridine (DMIP), 1,5,6-TMIP, 2-amino-1,6-dimethyl-furo[3,2-e]imidazo[4,5-b]-pyridine (IFP), (Borgen and others 2001), and PhIP (Skog and others 2000; Borgen and others 2001), while aqueous heating favors the formation of β-carbolines and IQx compounds (Skog and others 2000). This fact was shown when NaCl/sodium tripolyphosphate was added (Persson and others 2003b). During the drying processes and, for example, along the crust formation, the water activity, a_w, decreased. At a_w 0.44, the formation of HAAs in a model system is promoted. The formation of pyrazines and 2-methylpyrazines in the Maillard reaction reaches a maximum at a_w 0.75, although temperature is the main factor (Skog and others 1998a). In this way, in chicken breast samples high cooking temperature and high rate of dripping loss have a great impact on crust formation during pan frying and greatly favored the amount of PhIP formed. Also, high temperature and high rate of dripping loss are found to be the most favorable for the formation of PhIP (Persson and others 2002).

Factors that affect the formation of HAAs

Table 2 to 23 show HAA contents in foods and beverages. Dispersion of results are evident, and no data exist for some thermic HAAs, such as 1,5,6-TMIP, 3,5,6-TMIP, 4-CH_2OH-8-MeIQx, and for some pyrolytic ones, Phe-P-1, Orn-P-1, Cre-P-1, and Lys-P-1.

Physical variables, such as temperature, time, and method of cooking, affect significantly the mutagenic activity of cooked samples. It has been established that both the varieties and amounts of HAAs increase along with increasing temperature (Knize and others 1994c; Jackson and Hargraves 1995; Skog and others 1995; Augustsson and others 1997; Chiu and others 1998; Chen and Meng 1999; Thomson 1999; Skog and others 2000; Steinmann and Fischer 2000; Persson and others 2002, 2003b; Ristic and others 2004; Ahn and Grun 2005a; Bermudo and others 2005; Olsson

Table 2–Content of pyrolytic HAAs in meat extracts.

Sample	Method	Units	Glu-P-2	Glu-P-1	Harman	Norharman	AαC	MeAαC	Trp-P-2	Trp-P-1	Ref.
Beef extract	GC-NIC-MS-SIM	ng/g dry wt					<8.1	1.6 to 4.0	<6.4	0.3 to 5.0	(Skog and others 1998b)
Beef extract	LC-ES-MS	ng/g		8 ± 1	135 ± 12	61 ± 7	<0.1	<0.2	<0.2	13 ± 2	(Pais and others 1997a)
Beef extract	LC-APCI-MS	ng/g		10.1 ± 1.1	129.5 ± 16.8	74.0 ± 7.4	2.8 ± 0.38	<0.4	<0.8	<0.4	(Pais and others 1997b)
Beef extract	HPLC-ESI-MS-MS-SRM	ng/g					0.42 ± 0.03	<0.030			(Turesky and others 2007)
Beef extract	HPLC-FD-DAD	ng/g			377	93.8					(Totsuka and others 1999)
Beef extract	HPLC-ED or HPLC-FD	ng/g		nd	110 ± 20	53 ± 17	2 ± 1	nd	14 ± 5	nd	(Galcerán and others 1996)
Beef extract	HPLC-ED	ng/g		15.54	nq	nq		nq	nq		(Galcerán and others 1993)
Beef extract	HPLC-UV/FD	ng/g			6.65	56.0	nq	nq	2.03	4.02	(Martín-Calero and others 2007)
Chicken extract					nq	7.76	nq	nq	3.13	nq	
Meat juice, lyophilized, 175 °C/10 min	HPLC-FD-DAD	ng/g dry wt			300	210	nd				(Skog and others 2000)
Pan residue extract	MEKC-ED	ng/g	0.038	<0.002							(Olsson and others 1997)
Pan residue, 175 °C/45 min	HPLC-FD-DAD or GC-NIC-MS-SIM	ng/g dry wt			14.0	52.6	nd	nd	nd	1.7	(Solyakov and others 1999)
Bouillon, 100 °C/240 min	HPLC-UV/FD	ng/g			nq to 0.2	nq to 0.4	nd	nd	nd	nd	(Solyakov and Skog 2002)
Bouillon concentrated, commercial		ng/mL			8.4 ± 2	8.0 ± 1.7	nd	nd	nd	nd	
Bouillon concentrated	HPLC-FD-DAD or GC-NIC-MS-SIM	ng/g dry wt			3.3 to 21.9	7.9 to 22.3	nd	nd	nd	nd	(Solyakov and others 1999)
Bouillon cube, commercial	HPLC-UV/FD	ng/mL			4.6 ± 0.2	3.2 ± 2.3	nd	nd	nd	nd	(Solyakov and Skog 2002)
Soup cubes	HPLC-UV/FD	ng/g			nq to 3.92	nq to 65.6	nq	nq	nq	nq	(Martín-Calero and others 2007)
Chicken bouillon cubes	GC-EI-MS-SIM	ng/g				43.2 ± 5	<0.05	<0.07	<0.29	<0.35	(Casal and others 2004)
Process flavor	HPLC-FD-DAD or GC-NIC-MS-SIM	ng/g dry wt			<755	<176	<0.4	<20.3	nq	<1.4	(Solyakov and others 1999)
Processed food flavors	LC-APCI-MS-SIM	ng/g							nq		(Stavric and others 1997)
–	LC-APCI-IT-MS-MS Extraction method A	ng/g	<1.2	<1.7	240.7 ± 35.5	180.2 ± 20.5	nq	nq	<0.8	nq	(Toribio and others 2002)
	Extraction method B		<2.7	<4.6	314.5 ± 53.1	185.7 ± 14.8	nq	nq	<1.7	<3.1	
–	LC-APCI-IT-MS	ng/g	<7.9	<9.0	263	146	nq	nq	<1.9	<1.7	(Toribio and others 2000b)
–	LC-APCI-IT-MS	ng/g	<3.7 to 5.4	<4.7 to 9.1	264.0 to 360.3	118.7 to 158.6	nq	nq	<2.0 to 11.7	<2.4	(Toribio and others 2000c)

nd = nondetected; nq= nonquantified.

and others 2005; Shin 2005; Turesky and others 2007; Costa and others 2009; Friedman and others 2009; Liao and others 2009) and also with time (Jautz and others 2008). Measured values of the specific HAAs vary with the meat type, cut of meat, cooking method, and doneness level (Knize and others 1995; Sinha and others 1995; Knize and others 1998; Sinha and others 1998b; Shin 2005; Costa and others 2009). Other factors depending on the type of food are pH, amounts of precursors, presence of certain divalent ions, content of substances with enhancing or inhibiting effects, and types of amino acids (Skog 1993; RobbanaBarnat and others 1996; Jägerstad and others 1998; Skog and others 1998a; Thomson 1999; Skog and others 2000; Borgen and others 2001; Gu and others 2002; Persson and others 2003b; Ristic and others 2004; Olsson and others 2005; Shin 2005).

Effect of muscle types. Muscle types (ordinary and dark muscles), skin, and degrees of cooking also affect the formation of HAAs (Gu and others 2002) (Table 20 and 21). There are differences between dark and ordinary muscles in terms of HAA precursors. Thus, ordinary muscle contains higher amounts of moisture, total free amino acids, creatine, and other nitrogen compounds than dark muscle. Creatine is not detected in the skin. Skin contains mucus as a lubricant and mechanical protector, composed mostly of glycoproteins. Thermally treated dark muscle contains low levels of HAAs, and this can also be due to its location between skin and ordinary muscle, resulting in the indirect contact with a hot pan (Gu and others 2002).

Chicken meat is called white meat, in contrast to red meat, for example, beef and pork. In chicken meat, the main difference is the low fat content, although the amino acid pattern and the content of glucose and creatine also differ (Borgen and others 2001). PhIP and IFP seem to be formed more easily in chicken than in beef, pork, or fish during cooking, while the amount of other HAAs, such as 8-MeIQx, is generally lower in cooked chicken than in cooked beef and pork (Skog and others 1998a; Borgen and others 2001; Skog and Solyakov 2002). No HAAs were detected in fried chicken liver (Solyakov and Skog 2002). This can be due to the very low level of creatin(in)e detected in the raw chicken liver. The presence of HAAs in the skin can be explained by direct exposure to the heat source, while the skin acts as an insulating layer for the meat (Solyakov and Skog 2002). But both the amounts of HAAs and the weight loss of chicken legs with skin were less than those without skin under the same microwave conditions, and these

Table 3–Content of thermic HAAs in meat extracts.

Sample	Method	Units	PhIP	IQ	MeIQ	IQx	8-MeIQx	7,8-DiMeIQx	4,8-DiMeIQx	Others	Ref.
Beef extract	LC-ES-MS	ng/g	10 ± 1	<0.2	<0.3		<1.1		<0.2		(Pais and others 1997a)
Beef extract	LC-APCI-MS	ng/g	<0.4	<0.2	<0.2		<1.0		<1.4		(Pais and others 1997b)
Beef extract	HPLC-ESI-MS-MS-SRM	ng/g	1.82 ± 0.07	75.20 ± 0.23		3.06 ± 0.19	37.80 ± 0.61		6.20 ± 0.28	IQ[4,5-*b*]: 0.097 ± 0.009 Iso-IQx: 5.70 ± 0.17 Iso-MeIQx: 27.60 ± 0.81 7,9-DiMeIgIQx: 10.90 ± 2.97	(Turesky and others 2007)
Beef extract	HPLC-FD/ED	ng/g	nd	nd	nd		nd		nd		(Galcerán and others 1996)
Beef extract	HPLC-UV/FD	ng/g								7,9-DiMeIgIQx: 53	(Nukaya and others 1994)
Beef extract	HPLC-UV/FD	ng/g	7.42								(Martín-Calero and others 2007)
Beef extract	HPLC-ED	ng/g	nq	<0.74	5.77		5.07		<3.37		(Galcerán and others 1993)
Beef extract	HPLC-ED	ng/g		9.59	nd		35.91				(Van Dyck and others 1995)
Beef extract	GC-NICI-MS	ng/g	<0.1				0.6		<0.1		(Murray and others 1993)
Beef extract	CZE-UV	ng/g			10.4		9.3				(Puignou and others 1997)
Commercial	CZE-UV-DAD	ng/g	<0.73	<0.20			<0.05		<0.14		(Mardones and others 1998)
Chicken extract	HPLC-UV/FD	ng/g	nd								(Martín-Calero and others 2007)
Meat juice, lyophilized, 175 °C/10 min	HPLC-FD-DAD	ng/g dry wt	nd			nd	39				(Skog and others 2000)
Pan residue extract	MEKC-ED	ng/g		0.014	<0.0096		1.0	0.054	0.51	TriMeIQx: 0.021	(Olsson and others 1997)
Pan residue, 175 °C/45 min	HPLC-UV/FD	ng/g dry wt	nd	nd	nd	2.0	2.3	nd	1.3		(Solyakov and others 1999)
Vacuum dried	HPLC-UV or HPLC-ESI-MS	ng/g	nd			2.1	29.0	nd	4.8		(Fay and others 1997)
Microwaved			7.5			5.9	46.0	nd	6.2		
Bouillon, 100 °C/240 min	HPLC-UV/FD	ng/g	nd	nd	nd	nd	nd	nd	nd		(Solyakov and Skog 2002)
Bouillon concentrated, commercial		ng/mL	nd	nd	nd	nd	0.1	nd	nd		
Bouillon concentrated	HPLC-UV/FD	ng/g dry wt	nd	nd	nd	nd	nd	nd	nd		(Pais and others 1999)
Bouillon cube	HPLC-UV	ng/g					nd				(Johansson and Jägerstad 1994)
Bouillon cube, commercial	HPLC-UV/FD	ng/mL	nd	nd	nd	nd	nd	nd	nd		(Solyakov and Skog 2002)
Beef stock cubes	GC-NICI-MS	ng/g	0.3				0.6		0.3		(Murray and others 1993)
Soap cubes	HPLC-UV/FD	ng/g	nd								(Martín-Calero and others 2007)
Soap cubes	IP-HPLC-CEAD (Ion-pair chromatography with coulometric electrode array detection)	ng/g		<0.1 to 0.7			0.2 to 1.0	<0.14	<0.74 to 2.8		(Krach and Sontag 2000)
Veal cubes, fried	HPLC-UV	ng/g fresh wt	<0.1	<0.1	<0.1		<0.1 to 1.0	<0.1	<0.1		(Zimmerli and others 2001)
Chicken bouillon cubes	GC-EI-MS-SIM	ng/g	<0.12	<0.09	0.5 ± 0.2	<0.05	<0.05	<0.06	3.7 ± 0.7		(Casal and others 2004)
Chicken stock cubes	GC-NICI-MS	ng/g	nd				nd		nd		(Murray and others 1993)
Chicken flavor paste	HPLC-UDV or HPLC-ESI-MS	ng/g	nq			0.6	5.0	nd	nd		(Fay and others 1997)
Beef flavors	HPLC-FD-DAD	ng/g dry wt	nd	nd	nd		7.2 to 21.2		4.2		(Jackson and others 1994)
Process flavor	HPLC-UV/FD	ng/g dry wt	nq	<3.4	nq	<0.7	<13.8	<0.3	<2.9		(Solyakov and others 1999)
Processed food flavors	LC-APCI-MS-SIM	ng/g	nq	<0.6 to 9.6	<0.6		nq				(Stavric and others 1997)
–	LC-APCI-IT-MS-MS Extraction method A	ng/g	25.0 ± 3.0	31.3 ± 3.3	<1.1		40.5 ± 6.4	<0.9	16.4 ± 1.2	DMIP: <10.3	(Toribio and others 2002)
	Extraction method B		24.1 ± 3.2	36.5 ± 3.4	<2.4		40.2 ± 7.6	<1.5	18.1 ± 1.2	DMIP: <4.9	
–	LC-APCI-IT-MS	ng/g	27.1	32.5	<10.1		41.4	<2.9	9.7		(Toribio and others 2000b)
–	LC-APCI-IT-MS	ng/g	24.4 to 31.3	31.2 to 37.5	<6.5 to 10.2		39.6 to 46.1	<3.1 to 4.5	9.7 to 13.3		(Toribio and others 2000c)
–	LC-APCI-MS-MS-SRM	ng/g	0.09 to 5.97	0.11 to 4.76			0.92 to 45.51	<0.045 to 0.65	0.18 to 13.65	7,9-DiMeIgIQx: 22.55	(Guy and others 2000)

Table 4–Content of pyrolytic HAAs in beef meats.

Sample	Method	Units	Glu-P-2	Glu-P-1	Harman	Norharman	AαC	MeAαC	Trp-P-2	Trp-P-1	Ref.
Cooked beef											
275 °C/30 min	HPLC-FD-DAD	ng/g fresh wt			<0.1	11.5					(Pais and others 1999)
180 to 220 °C/10 to 30 min, lyophilized	HPLC-API-ES-MS	ng/g					nq to 62	nq to 6.4	nq	4 to 150	(Messner and Murkovic 2004)
Blackened beef, or top sirloin steak, 100 °C/10 to 17 min	HPLC-FD-DAD	ng/g					<0.1				(Knize and others 1997b)
Meat loaf, roasted, 175 °C/45 min, pan residue	GC-NIC-MS-SIM	ng/g dry wt					0.28	<0.1 ng	<2 ng	0.08	(Skog and others 1998b)
Grilled/roasted beef											
longissimus dorsi muscle, grilled, 220 °C, internal temperature 65 or 80 °C; 1, 14, or 28 d of aging	HPLC-API-ESI-MS-SIM	ng/g			nd	nd					(Polak and others 2009a)
Grilled, 200 to 250 °C/15 min	HPLC-ED or HPLC-DAD	ng/g		<4.2							(Rivera and others 1996)
Grilled, 185 to 273 °C/9 to 41 min	HPLC-FD-DAD	ng/g			5.39	7.34					(Totsuka and others 1999)
Roast beef, oven-broiled, 175 to 185 °C/4 to 24 min					4.03	5.0					
Broiled, 6 min					169	795					
Fillet, grilled, 200 °C/15 min	LC-ESI-MS-MS-MRM	ng/g					nq	nq	nq	nq	(Khan and others 2008)
Roast beef loin, oven-broiled, 200 °C/40 min							nq	nq	nq	0.010 ± 0.004	
Meat patties, grilled	HPLC-FD-DAD, HPLC-TS-MS-SIM	ng/g					<0.5				(Gross and others 1993)
Meat scrapings, grilled							77				
Beef steak, grilled, 180 to 210 °C/4 min	LC-IT-MS-MS	ng/g	<0.3	<0.3	5.9 ± 1.2	21.2 ± 3.1	0.33 ± 0.25	0.15 ± 0.12	<0.1	0.35 ± 0.2	(Toribio and others 2007)
Beef steak, grilled, well done			<0.3	<0.3	1.90 to 5.31	2.42 to 12.9	<0.18 to 1.4	nq	<0.1	nd to 0.35	
Beef steak, grilled, 250 to 270 °C/20 min	LC-AP-ESI-MS-SIM	ng/g	<2.71	<3.13			<1.57		<0.95	<1.45	(Kataoka and Pawliszyn 1999)
Barbecued, 230 to 300 °C/10 min	HPLC-ESI-MS-MS-SRM	ng/g					2.80 to 7.75	0.088 to 0.29			(Turesky and others 2007)
Barbecued, 200 °C/12 min or 240 °C/7 min	HPLC-FD-DAD	ng/g			0.09 to 2.36	0.32 to 7.18			nd to 0.26		(Abdulkarim and Smith 1998)
Crusts of barbecued beef, 200 to 240 °C/7 to 30 min					0.52 to 28.6	1.30 to 30.0			nd to 1.59		
Roasted, commercial	LC-ESI-MS	ng/g	nd	nd	nd to 240.0	nd to 205.0	nd	nd to 15.6	nd	nd	(Jo and others 2008)
Rib, grilled, commercial			nd	nd	nd to 80.0	nd to 176.0	nd	nd to 13.2	nd to 3.8	nd	
Roast beef, gravy, oven-roasted, 160 °C/96 to 182 min, rare done at well done	LC-ESI-MS-MS-SRM	ng/g					<0.03	<0.03			(Ni and others 2008)
Fried beef											
190 °C/10 min or 230 °C/15 min	HPLC-FD-DAD	ng/g			0.15 to −0.83	0.50 to 2.60			nd to 0.38		(Abdulkarim and Smith 1998)
Crusts, 150 to 230 °C/6 to 30 min					0.23 to −1.70	0.96 to 5.65			nd to 1.70		
277 °C/12 min	HPLC-FD-DAD	ng/g					21.0		nd		(Thiebaud and others 1994)

(*Continued*)

Table 4–(*Continued*)

Sample	Method	Units	Glu-P-2	Glu-P-1	Harman	Norharman	AαC	MeAαC	Trp-P-2	Trp-P-1	Ref.
Pan-fried, 176 to 191 °C/4 to 33 min	HPLC-FD-DAD	ng/g			3.80	12.5					(Totsuka and others 1999)
270 °C/3 to 7 min	HPLC-FD-DAD HPLC-TS-MS-SIM	ng/g			nd	3.3					(Gross and others 1993)
270 °C/>15 min					7.0	1.2					
Beef steak, 190 °C/6 to 13 min					3 to 4.8	8.7 to 19.3					
Beef steak, fried, 180 to 200 °C/8 min	HPLC-FD/DAD	ng/g	nd	nd			19 ± 2.5	nq	nq	nq	(Melo and others 2008b)
Beef steak, fried, 186 to 191 °C/16 to 33 min, medium at very well done	LC-ESI-MS-MS-SRM	ng/g					<0.03	<0.03			Ni and others 2008)
Beef steak, fried, 180 to 200 °C/12 min	HPLC-ESI-IT-MS-MS	ng/g			4.3 ± 0.3	6.0 ± 0.8	<0.1	0.7 ± 0.1	<0.3	<0.5	(Busquets and others 2008)
Beef steak, coated-fried, 180 to 200 °C/14 min					6.7 ± 0.4	15.4 ± 0.8	nd	nd	<0.1	<0.1	
Beef steak, griddled, 180 to 210 °C/4 min	LC-ES-MS-MS-MRM	ng/g	<0.1	<0.1	5.3 ± 0.8	41.2 ± 7.4	0.5 ± 0.1	0.4 ± 0.04	<0.02	0.6 ± 0.1	(Busquets and others 2004)
Chop, roasted at 90 °C/20 min, fried 6 min	LC-ESI-MS-MS-MRM	ng/g					nq	nq	nq	0.012 ± 0.004	(Khan and others 2008)
Patties, fried, 230 °C/3 min	HPLC-FD-DAD	ng/g			1.5 ± 0.2	2.1 ± 0.3					(Jautz and others 2008)
	HPTLC-FD-DAD				1.4 ± 0.2	3.7 ± 0.9					
Patties, fried, 230 °C/4 min, 30 s	HPLC-FD-DAD				3.5 ± 0.4	5.0 ± 0.3					
	HPTLC-FD-DAD				3.1 ± 0.4	6.8 ± 0.7					
Patties, fried, 230 °C/6 min	HPLC-FD-DAD				8.9 ± 1.0	10.4 ± 0.9					
	HPTLC-FD-DAD				10.3 ± 2.7	11.3 ± 3.3					
Ground beef patties, 300 °C/6 min	HPLC-ESI-MS-MS-SRM	ng/g					3.32 ± 0.90	0.14 ± 0.06			(Turesky and others 2007)
Ground beef patties, 150 to 190 °C/10 to 12 min							<0.030	<0.030			
Ground beef scrapings, 150 to 180 °C/10 min							2.89 ± 0.60	0.76 ± 0.24			
Ground beef patties, 150 to 230 °C/4 to 20 min	HPLC-FD-DAD	ng/g					nd		nd	nd	(Knize and others 1994c)
Ground beef patties, oven-broiled, 180 to 189 °C/6 to 15 min, rare to well done	LC-ESI-MS-MS-SRM	ng/g					nd	nd			(Ni and others 2008)
Ground beef patties, oven-broiled, 191 °C/20 min, very well done							0.11 ± 0.11	nd			
Ground beef patties, fried, 180 to 191 °C/10 to 20 min, medium at very well done							nd	nd			

(*Continued*)

Table 4–(*Continued*)

Sample	Method	Units	Glu-P-2	Glu-P-1	Harman	Norharman	AαC	MeAαC	Trp-P-2	Trp-P-1	Ref.
Ground beef patties, 230 °C/5 min, marinated with onion, garlic, and lemon juice	HPLC-UV	ng/g			2.9 to 21.5	0.76 to 13.5					(Gibis 2007)
Kebab, medium-done, fast food	HPLC-ESI-MS-SIM	ng/g			nd to 0.75	nd to 0.71	0.12 to 0.52	0.06 to 0.16	0.45 to 0.69	nd	(Borgen and Skog 2004)
Nuggets, deep-fried, commercial	HPLC-ESI-MS-MS-SRM	ng/g	<0.1	nq							(Richling and others 1998)
Meat sauce, fried, 175 to 225 °C/6 min	HPLC-UV/FD	ng/g fresh wt			nq	nq	nq	nq	nq to 0.6	0.3 to 0.7	(Skog and others 1997)
Meat sauce, fried, 175 to 200 °C/6 min	HPLC-FD-DAD	ng/g			nq	nq			0.6	0.3 to 0.7	(Skog and others 1995)

weight losses were higher than those of fried chicken legs (Chiu and others 1998) (Table 14 and 15).

A gene, the RN^- allele has been shown to influence significantly the production and meat quality characteristics in certain pigs. This dominant allele causes higher glycogen content in glycolytic muscles than in other pig breeds and noncarriers (normal pigs) of this allele (Olsson and others 2002). The increased glycogen concentration leads to an increased level of residual glycogen, namely, glycogen, glucose, and glucose-6-phosphate in the meat after slaughter. The meat from RN^- carriers contains, on average, 4-fold higher concentrations of residual glycogen compared with meat from noncarriers. A bimodal distribution of PhIP content with 2 extreme groups of values is found when the level of PhIP is plotted against residual glycogen. It is clearly seen that the increase of residual glycogen concentrations reduces significantly the yield of total HAAs, in particular PhIP, and this gives as a result about 50% lower amounts of total HAAs in cooked meat from carriers of the RN^- allele than in cooked meat from normal pigs (Table 10 and 11). Cooked meat from RN^- carriers is significantly darker than that of noncarriers and, surprisingly, there is no correlation (or in the case of PhIP, a negative correlation) between the amount of HAAs and the degree of surface browning. The observation that a higher degree of surface browning of fried meat does not necessarily mean higher levels of HAAs is also important for dietary assessment studies (Olsson and others 2002, 2005). On the other hand, with independence of the genotype, feeding regime, or sex does not significantly affect the chemical composition or the formation of HAAs (Olsson and others 2002). Thus, for the 1st time the effect of feed supplementation on the formation of HAAs in fried pork was investigated by Pfau and others (2006). Groups of Duroc and Landrace pigs, both noncarriers of RN^- allele, received feed supplemented with creatine monohydrate for 5 d prior to slaughter. Creatine phosphate levels increased in the meat with creatine monohydrate supplementation, but this was only significant in meat from Duroc pigs. No significant difference was observed with regard to creatine content, measured as creatine phosphate or content of HAAs upon frying measured as mutagenicity of extracts (Pfau and others 2006).

Model systems have been used extensively to simplify the meat matrix. Thus, diverse parallel reactions are eliminated and the reactions of interest are more easily studied. It is also advisable that conditions be defined and realistic (Jackson and Hargraves 1995; Wild 1996; Arvidsson and others 1999; Pais and others 1999; Skog and others 2000). The model system allows to obtain reproducible data on concentration changes induced by heating. In this way, in studies involving the frying of meat, many parameters, such as heat and mass transfer, vaporization of water, and crust formation, are difficult to control. However, these parameters can be studied in model systems in which reaction conditions are reproducible and well controlled (Messner and Murkovic 2004). The formation of HAAs in model systems has been reviewed by Murkovic (2004b).

Effects of cooking temperature and time. The formation of HAAs is highly dependent on time and temperature of cooking (Gross and Grüter 1992; Skog 1993; Knize and others 1994c; Jackson and Hargraves 1995; Johansson and others 1995b; Skog and others 1995; Wild 1996; Arvidsson and others 1997; Abdulkarim and Smith 1998; Murkovic and others 1998; Arvidsson and others 1999; Chen and Meng 1999; Thomson 1999; Balogh and others 2000; Chen and others 2000; Krul and others 2000; Murkovic and Pfannhauser 2000; Salmon and others 2000; Skog and others 2000; Zimmerli and others 2001; Persson and others 2002; Sinha 2002; Solyakov and Skog 2002; Persson and others 2003b; Bordas and others 2004; Messner and Murkovic 2004; Murkovic 2004a; Ristic and others 2004; Ahn and Grun 2005a; Bermudo and others 2005; Olsson and others 2005; Shin 2005; Pfau and others 2006; Turesky and others 2007; Ni and others 2008; Costa and others 2009; Liao and others 2009; Polak and others 2009b). Levels of HAAs are low or nondetectable in foods fried at 150 °C, but a sharp increase is detected at higher cooking temperatures above 190 °C (Jackson and Hargraves 1995; Johansson and others 1995b; Abdulkarim and Smith 1998; Balogh and others 2000). Generally, if the cooking temperature exceeds 200 °C, the total level of HAAs increases drastically (Jackson and Hargraves 1995; Wild 1996; Skog and others 1997; Abdulkarim and Smith 1998; Balogh and others 2000; Turesky and others 2007). The formation of PhIP, AαC, and the β-carbolines is markedly influenced by cooking temperature and is clearly nonlinear (Gross and Grüter 1992; Bordas and others 2004; Olsson and others 2005). Norharman and 4,8-DiMeIQx are more sensitive to temperature change, whereas 8-MeIQx and AαC are less sensitive (Ahn and Grun 2005a).

Temperatures lower than 200 to 225 °C do not form detectable concentrations of pyrolytic HAAs in meats; however, at higher temperatures (>250 °C), the concentrations of the IQ-type compounds decrease in the model systems (Felton and others 1999; Pais and others 1999). Therefore, no AαC, MeAαC, Trp-P-1, Trp-P-2, Glu-P-1, or Glu-P-1 were found in samples baked at 225 °C. These results are in agreement with those obtained by other authors who found decreased amounts of some HAAs and the mutagenic activity in model systems and in meats at long

Table 5–Content of thermic HAAs in beef meats.

Sample	Method	Units	PhIP	IQ	MeIQ	IQx	8-MeIQx	7,8-DiMeIQx	4,8-DiMeIQx	Others	Ref.
Cooked beef											
–	HPLC-FD-DAD	ng/g	<0.1 to 182				<0.1 to 3.0		<0.1 to 0.77	IFP: <0.1 to 46 DMIP: <0.1 to 7.2 TMIP: <0.1 to 1.5	(Pais and others 2000)
275 °C/30 min	HPLC-FD-DAD	ng/g fresh wt	1.2 ± 0.6	nd	nd	nd	1.43 ± 0.08		0.2 ± 0.05	IFP: 0.2 ± 0.06 DMIP: nd TMIP: nd 4-MeIQx: nd	(Felton and others 1999; Pais and others 1999)
275 °C/30 min, meat drippings			5.4 ± 3.0	nd	nd	0.2 ± 0.03	6.6 ± 3.4		1.6 ± 1.5	IFP: 13.2 ± 8.2 DMIP: 13.4 ± 6.7 TMIP: 0.8 ± 0.3 4-MeIQx: nd	
180 to 220 °C/10 to 30 min, lyophilized	HPLC-API-ES-MS	ng/g	170 to 420	68 to 94			nq to 30		3 to 95	DMIP: 19 to 40	(Messner and Murkovic 2004)
Steak, broiled, 178 to 185 °C/10 to 24 min	HPLC-FD-DAD	ng/g	2.1 to 7.1	<0.2	<0.2		<0.2 to 1.7		<0.2		(Sinha and others 1998b)
Blackened beef, 100 °C/10 to 17 min	HPLC-FD-DAD	ng/g	1.0	<0.1			0.48		<0.1		(Knize and others 1996, 1997b)
Top sirloin steak, 100 °C/10 to 17 min			13	<0.1			0.87		<0.1		(Knize and others 1997b)
Beef collar pan-fried, 150 to 160 °C/20 min, simmered 90 to 95 °C/1 h medium done	HPLC-DAD	ng/g	1.9	nq	nq		nq		nq		(Warzecha and others 2004)
Ragout, fried/cooked	HPLC-UV	ng/g fresh wt	<0.1 to 6.0	<0.1	<0.1		<0.1 to 0.8	<0.1	<0.1 to 1.0		(Zimmerli and others 2001)
Roast beef, fried/cooked			<0.1 to 3.7	<0.1	<0.1		1.0	<0.1	<0.1		
Meat balls (commercial)	HPLC-UV	ng/g	0.6	0.2	0.3		0.7		0.2		(Johansson and Jägerstad 1994)
Meat balls, fried, 150 to 225 °C/6.5 to 9 min	HPLC-FD-DAD	ng/g	nd to 0.1	nd			nd to 0.8	nd to 0.3			(Skog and others 1995)
Meat balls, fried, pan residue 150 to 225 °C/6.5 to 9 min			0.03 to 0.5	0.05			0.02 to 0.7	0.02 to 0.1			
Minced, 100 to 90 °C/40 min	HPLC-FD-DAD	ng/g	nd				nd		nd		(Sinha and others 1994)
Grilled/roasted beef longissimus *dorsi* muscle, grilled, 220 °C; internal temper. 65 °C; no aging	HPLC-API-ESI-MS-SIM	ng/g	nd	nd	nd	nd	0.19 ± 0.03		nd		(Polak and others 2009a)
"; internal temperature 65 °C; 14 d of aging			nd	nd	nd	nd	0.36 ± 0.06		nd		
"; internal temperature 65 °C; 28 d of aging			0.05 ± 0.02	nd	nd	nd	0.34 ± 0.09		nd		
"; internal temperature 80 °C; no aging			0.05 ± 0.01	nd	nd	nd	0.15 ± 0.03		nd		
"; internal temperature 80 °C; 14 d of aging			0.11 ± 0.01	nd	nd	nd	0.15 ± 0.06		nd		
"; internal temperature 80 °C; 28 d of aging			0.14 ± 0.02	nd	nd	nd	0.14 ± 0.09		nd		
200 to 250 °C/15 min	HPLC-ED or HPLC-DAD	ng/g		7 ± 2	8 ± 2		4 ± 2				(Rivera and others 1996)
–	HPLC-UV or HPLC-ESI-MS	ng/g	14			1.5	6.0	0.2	1.2		(Fay and others 1997)
Minced	HPLC-FD-DAD	ng/g	0.52				0.14	<0.1	<0.1	IFP: <0.1	(Wong and others 2005)
Broiled	HPLC-UV	ng/g	21							4′-OH-PhIP: 21	(Kurosaka and others 1992)
Fillet, grilled, 200 °C/15 min	LC-ESI-MS-MS-MRM	ng/g	1.42 ± 0.23	nq	nq		1.16 ± 0.17		0.12 ± 0.02	DMIP: 0.44 ± 0.10	(Khan and others 2008)
Roast beef loin, oven-broiled, 200 °C/40 min			1.04 ± 0.09	nq	nq		0.21 ± 0.05		0.36 ± 0.04	DMIP: 0.55 ± 0.10	
Meat patties	HPLC-FD-DAD, HPLC-TS-MS-SIM	ng/g	0.8 to 3.2				0.8 to 3.2		<0.5		(Gross and others 1993)
Meat scrapings			144				29		4		

(*Continued*)

Table 5–(*Continued*)

Sample	Method	Units	PhIP	IQ	MeIQ	IQx	8-MeIQx	7,8-DiMeIQx	4,8-DiMeIQx	Others	Ref.
Veal sausage, grilled	HPLC-UV	ng/g fresh wt	<0.1 to 1.6	<0.1	<0.1		<0.1 to 0.5	<0.1	<0.1		(Zimmerli and others 2001)
Mixed grill			1.0	<0.1	<0.1		1.4	<0.1	<0.1		
Steak			<0.1	<0.1	<0.1		0.7 to 1.0	<0.1	<0.1		
Beef steak, grilled, 180 to 210 °C/4 min	LC-IT-MS-MS	ng/g	6.99 ± 0.81	<0.1	<0.1		2.87 ± 0.69	<0.1	1.27 ± 0.28	DMIP: nq	(Toribio and others 2007)
Beef steak, grilled, well done			1.73 to 3.49	<0.1	<0.1		0.78 to 1.70	<0.1	0.28 to 0.72	DMIP: nd to nq	
Beef steak, charbroiled	HPLC-FD-DAD	ng/g	5.7 to 15	<0.1	<0.1		1.1 to 2.4	<0.1 to 0.4			(Knize and others 1998)
Beef steak, 225 °C/12 min	GC-NICI-MS	ng/g	0.6				0.5		0.1		(Murray and others 1993)
Beef steak, 250 to 270 °C/20 min	LC-AP-ESI-MS-SIM	ng/g	1.18 ± 0.05	0.25 ± 0.01	<0.55		1.15 ± 0.10	<0.92	<1.33		(Kataoka and Pawliszyn 1999)
Barbecued, 230 to 300 °C/10 min	HPLC-ESI-MS-MS-SRM	ng/g		0.036 to 0.129		<0.03 to 0.20	0.527 to 1.600		0.119 to 0.431	IQ[4,5-*b*]: 0.046 to 0.172 Iso-IQx: 0.154 to 1.070 Iso-MeIQx: 1.180 to 6.490 7,9-DiMeIgIQx: 0.063 to 0.569	(Turesky and others 2007)
Beef steak, 249 to 273 °C/16 to 41 min	HPLC-FD-DAD	ng/g	2.5 to 30.0	<0.2	<0.2		0.2 to 2.7		<0.2		(Sinha and others 1998b)
Barbecued, 200 °C/12 min or 240 °C/7 min	HPLC-FD-DAD	ng/g	0.31 to 1.40				nd to 1.61				(Abdulkarim and Smith 1998)
Crusts of barbecued beef, 200 to 240 °C/7 to 30 min			0.10 to 4.20				0.27 to 4.00				
Meat loaf, roasted, 150 °C/55 min	HPLC-UV/FD	ng/g	0.3				0.1		<4 ng		(Skog and others 1997)
Meat loaf, roasted, 150 °C/55 min, pan residues			<0.4 ng				0.04		0.03		
Roasted ox, ready to eat	HPLC-ESI-MS-MS-SRM	ng/g	0.5	<0.1	<0.1		5.2		0.4		(Richling and others 1998)
Roasted, commercial	LC-ESI-MS	ng/g	nd to 32.4	nd	nd		nd to 17.5	nd to 2.8	nd to 3.4	TriMeIQx: nd	(Jo and others 2008)
Rib, grilled, commercial			nd	nd	nd		nd to 18.2	nd	nd	TriMeIQx: nd	
Roast beef gravy, oven-roasted; 160 °C/96 to 182 min	HPLC-FD-DAD	ng/g	<0.2 to 4.1	<0.2	<0.2		1.0 to 7.1		<0.2 to 1.1		(Sinha and others 1998b)
Roast beef gravy, oven-roasted, 160 °C/96 min, rare done	LC-ESI-MS-MS-SRM	ng/g	0.30 ± 0.01	<0.03		0.13 ± 0.02	1.10 ± 0.05		0.23 ± 0.00	IFP: 0.21 ± 0.01 IQ[4,5-*b*]: 0.15 ± 0.05 IgQx: 0.44 ± 0.03 7-MeIgQx: 3.78 ± 0.26 6,7-DiMeIgQx: 0.23 ± 0.01 7,9-DiMeIgQx: 0.73 ± 0.06	(Ni and others 2008)
";160 °C/120 min, medium done			0.06 ± 0.01	0.21 ± 0.11		0.13 ± 0.02	1.08 ± 0.08		0.21 ± 0.01	IFP: 0.11 ± 0.01 IQ[4,5-*b*]: 0.11 ± 0.01 IgQx: 0.34 ± 0.00 7-MeIgQx: 2.74 ± 0.07 6,7-DiMeIgQx: <0.03 7,9-DiMeIgQx: 0.50 ± 0.04	
";160 °C/182 min, well done			3.41 ± 0.17	0.32 ± 0.03		0.78 ± 0.03	8.43 ± 0.16		1.40 ± 0.10	IFP: 3.24 ± 0.29 IQ[4,5-*b*]: 0.04 ± 0.01 IgQx: 2.77 ± 0.10 7-MeIgQx: 29.52 ± 2.81 6,7-DiMeIgQx: 0.90 ± 0.05 7,9-DiMeIgQx: 6.09 ± 0.64	
Fried beef											
180 °C/20 min	HPLC-ECD/FD-DAD	ng/g	5.48	10.2	2.46		13.2		2.26		(Murkovic and others 1998)
180 °C/20 min with spices			reduced in (0 to 46)%	reduced in (0 to 68)%	reduced in (23 to 60)%		reduced in (15 to 62)%		reduced in (0 to 61)%		
190 °C/10 min or 230 °C/15 min	HPLC-FD-DAD	ng/g	nd to 1.09				nd to 1.11				(Abdulkarim and Smith 1998)
Crusts, 150 to 230 °C/6 to 30 min			0.15 to 3.13				nd to 2.34				
277 °C/12 min	HPLC-FD-DAD	ng/g	67.5	nd			16.4		4.5		(Thiebaud and others 1994)

(*Continued*)

Table 5–(*Continued*)

Sample	Method	Units	PhIP	IQ	MeIQ	IQx	8-MeIQx	7,8-DiMeIQx	4,8-DiMeIQx	Others	Ref.
Pan fried, well done	HPLC	ng/g	0.59				0.79		0.24	IFP: 0.10	(Keating and others 2000)
Pan fried, very well done			0.62				1.87		0.45	IFP: 0.14	
Stir-fried	HPLC-FD-DAD	ng/g	nd				<0.1	nd	nd	IFP: nd	(Wong and others 2005)
Beef, 14 min	CZE-UV-DAD	ng/g	<0.22	2.0 ± 1.1			8.7 ± 7.9		4.1 ± 2.6		(Mardones and others 1998)
Beef steak, 14 min			<0.22	12.5 ± 4.5			5.0 ± 2.1		1.8 ± 0.6		
Beef steak, 125 °C/20 min	HPLC-FD	ng/g	1.5 ± 0.23	<1	<1		1.5 ± 0.02				(Krul and others 2000)
Beef steak, coated-fried 180 to 200 °C/14 min	LC-IT-MS-MS	ng/g	0.2							4′-OH-PhIP: <0.08	(Busquets and others 2007)
Beef steak, pan-fried 180 to 200 °C/12 min			1.1							<0.08	
Beef steak, fried, 180 to 200 °C/8 min well done	HPLC-FD/DAD	ng/g	33.8 ± 5.5	nd	nd	nd	3.6 ± 0.5	nd	1.3 ± 0.7	TriMeIQx: nd	(Melo and others 2008b)
Beef steak, fried, 186 °C/16 min, medium done	LC-ESI-MS-MS-SRM	ng/g	1.77 ± 0.04	0.20 ± 0.09		0.16 ± 0.01	1.43 ± 0.06		0.33 ± 0.01	IFP: 0.34 ± 0.03 IQ[4,5-*b*]: 0.16 ± 0.02 IgQx: 0.76 ± 0.05 7-MeIgQx: 4.05 ± 0.53 6,7-DiMeIgQx: 0.23 ± 0.04 7,9-DiMeIgQx: 0.62 ± 0.09	(Ni and others 2008)
Beef steak, fried, 189 °C/26 min, well done			5.08 ± 0.25	0.19 ± 0.01		0.31 ± 0.01	3.17 ± 0.08		0.73 ± 0.01	IFP:1.26 ± 0.08 IQ[4,5-*b*]: 0.49 ± 0.14 IgQx: 1.55 ± 0.06 7-MeIgQx: 12.31 ± 0.81 6,7-DiMeIgQx: 0.28 ± 0.04 7,9-DiMeIgQx: 2.92 ± 0.12	
Beef steak, fried, 191 °C/33 min, very well done			12.46 ± 0.17	0.28 ± 0.03		0.69 ± 0.05	6.50 ± 0.23		2.06 ± 0.04	IFP: 4.03 ± 0.14 IQ[4,5-*b*]: 0.16 ± 0.00 IgQx: 4.03 ± 0.02 7-MeIgQx: 23.65 ± 1.73 6,7-DiMeIgQx: 0.68 ± 0.08 7,9-DiMeIgQx: 6.31 ± 0.29	
Beef steak, fried, 180 to 200 °C/12 min	HPLC-ESI-IT-MS-MS	ng/g	1.1 ± 0.3		nd		0.8 ± 0.4	nd	0.7 ± 0.4	DMIP: 1.1 ± 0.8 4′-OH-PhIP: <0.08	(Busquets and others 2007, 2008)
Beef steak, coated-fried, 180 to 200 °C/14 min			0.2 ± 0.1		nd		0.3 ± 0.1	nd	nd	DMIP: <0.3 4′-OH-PhIP: <0.08	
Beef steak, griddled, 180 to 210 °C/4 min	LC-ESI-MS-MS-MRM	ng/g	4.8 ± 0.6	<0.04	<0.04		2.9 ± 0.4	<0.1	1,1 ± 0.1	DMIP: <0.01	(Busquets and others 2004)
Beef steak, griddled	HPLC-FD-DAD	ng/g	6.8 to 10				1.7 to 2.4		<0.1 to 0.4		(Knize and others 1998)
–	HPLC-UV	ng/g fresh wt	<0.1 to 4.3	<0.1	<0.1		<0.1 to 1.5	<0.1	<0.1		(Zimmerli and others 2001)
Minute steak			<0.1 to 3.3	<0.1	<0.1		<0.1 to 0.6	<0.1	<0.1		
Minute steak, 150 to 225 °C/2.5 to 4 min	HPLC-FD-DAD	ng/g	0.02 to 12.7	nd			nd to 6.2		nd to 2.7		(Skog and others 1995)
Minute steak, 150 to 225 °C/2.5 to 4 min, pan residue			0.2 to 82.4	0.1			0.1 to 23.3		0.1 to 4.1		
Chop, roasted at 90 °C/20 min, fried 6 min	LC-ESI-MS-MS-MRM	ng/g	0.08 ± 0.02	0.011 ± 0.004	0.011 ± 0.004		0.020 ± 0.007		0.010 ± 0.003	DMIP: 0.08 ± 0.02	(Khan and others 2008)
Beef Cajun style, blackened	HPLC-FD-DAD	ng/g	1.0				0.48		<0.1		(Knize and others 1997a, 1997b)
Top sirloin steak, well done			13				0.87		<0.1		(Knize and others 1996, 1997a, 1997b)
Sirloin steak, 186 to 191 °C/15 to 33 min	HPLC-FD-DAD	ng/g	1.9 to 23.2	<0.2	<0.2		1.3 to 8.2		<0.2 to 1		(Sinha and others 1998b)
Sirloin steak, 150 to 225 °C/7 min	HPLC-FD-DAD	ng/g	0.06 to 1.8	nd to 0.04			0.02 to 1.6		0.02 to 0.6		(Skog and others 1995)
Sirloin steak, pan residue 150 to 225 °C/7 min			0.1 to 6.3	nd			0.07 to 3.3		nd		

(*Continued*)

Table 5–(*Continued*)

Sample	Method	Units	PhIP	IQ	MeIQ	IQx	8-MeIQx	7,8-DiMeIQx	4,8-DiMeIQx	Others	Ref.
Kebab, medium-done, fast-food	HPLC-ESI-MS-SIM	ng/g	0.22 to 0.25			0.16 to 0.40	0.14 to 0.24	0.16	0.09 to 0.22		(Borgen and Skog 2004)
Beef minced	GC-NICI-MS	ng/g	16.4				2.2		0.7		(Murray and others 1993)
Beef minced, 250 °C/22 min	HPLC-FD-DAD	ng/g	32.8				9.0		2.1		(Sinha and others 1994)
Patties, fried, 230 °C/3 min	HPLC-FD-DAD	ng/g	0.2 ± 0.02				1.0 ± 0.4		nd		(Jautz and others 2008)
	HPTLC-FD-DAD		3.2 ± 1.5				1.7 ± 0.5		1.1 ± 0.5		
Patties, fried, 230 °C/4 min, 30 s	HPLC-FD-DAD		0.9 ± 0.1				2.0 ± 0.3		0.4 ± 0.1		
	HPTLC-FD-DAD		9.9 ± 2.4				1.7 ± 0.3		1.4 ± 0.3		
Patties, fried, 230 °C/6 min	HPLC-FD-DAD		3.6 ± 0.8				4.8 ± 1.2		1.3 ± 0.1		
	HPTLC-FD-DAD		33.1 ± 9.7				4.8 ± 0.8		3.0 ± 0.3		
Ground beef patties, 300 °C/6 min	HPLC-ESI-MS-MS-SRM	ng/g	15.20 ± 2.90	0.260 ± 0.043		0.39 ± 0.08	5.310 ± 0.715		1.430 ± 0.345	IQ[4,5-*b*]: 1.420 to 0.288 Iso-IQx: 0.154 to 1.070 Iso-MeIQx: 13.80 ± 2.230 7,9-DiMeIgIQx: 1.020 ± 0.345	(Turesky and others 2007)
Ground beef patties, 150 to 190 °C/10 to 12 min			0.161 to 0.426	<0.03 to 0.040		0.12 to 0.26	1.450 to 3.720		0.175 to 0.588	IQ[4,5-*b*]: 0.349 ± 0.021 Iso-IQx: 0.154 to 1.070 Iso-MeIQx: 3.790 ± 0.602 7,9-DiMeIgIQx: 0.084 to 0.549	
Ground beef scrapings, 150 to 180 °C/10 min			82.50 ± 0.56	1.940 ± 0.095		6.38 ± 0.45	62.60 ± 3.42		15.00 ± 0.18	IQ[4,5-*b*]: 3.300 ± 0.404 Iso-IQx: 12.50 ± 2.570 Iso-MeIQx: 119.00 ± 19.40 7,9-DiMeIgIQx: 7.670 ± 3.690	
Ground beef patties, oven-broiled, 180 °C/6 min, rare done	LC-ESI-MS-MS-SRM	ng/g	<0.03	<0.03		<0.03	<0.03		<0.03	IFP: <0.03 IQ[4,5-*b*]: 0.26 ± 0.06 IgQx: <0.03 7-MeIgQx: <0.03 6,7-DiMeIgQx: <0.03 7,9-DiMeIgQx: <0.03	(Ni and others 2008)
Ground beef patties, oven-broiled, 186 °C/10 min, medium done			<0.03	0.05 ± 0.01		<0.03	<0.03		<0.03	IFP: <0.03 IQ[4,5-*b*]: 0.12 ± 0.01 IgQx: <0.03 7-MeIgQx: 0.31 ± 0.04 6,7-DiMeIgQx: 0.05 ± 0.00 7,9-DiMeIgQx: <0.03	
Ground beef patties, oven-broiled, 189 °C/15 min, well done			0.06 ± 0.04	0.06 ± 0.09		<0.03	0.02 ± 0.01		0.01 ± 0.00	IFP: <0.03 IQ[4,5-*b*]: 0.39 ± 0.55 IgQx: 0.03 ± 0.01 7-MeIgQx: 0.10 ± 0.02 6,7-DiMeIgQx: <0.03 7,9-DiMeIgQx: 0.02 ± 0.00	
Ground beef patties, oven-broiled, 191 °C/20 min, very well done			1.23 ± 0.30	0.10 ± 0.18		0.06 ± 0.01	0.38 ± 0.20		0.10 ± 0.00	IFP: 0.14 ± 0.01 IQ[4,5-*b*]: 0.15 ± 0.09 IgQx: 0.30 ± 0.02 7-MeIgQx: 1.35 ± 0.05 6,7-DiMeIgQx: <0.03 7,9-DiMeIgQx: 0.12 ± 0.02	
Ground beef patties, fried, 180 °C/10 min, medium done			0.70 ± 0.10	0.10 ± 0.10		0.10 ± 0.00	1.00 ± 0.10		0.20 ± 0.00	IFP: 0.10 ± 0.00 IQ[4,5-*b*]: 0.30 ± 0.10 IgQx: 0.50 ± 0.00 7-MeIgQx: 2.40 ± 0.20 6,7-DiMeIgQx: 0.20 ± 0.10 7,9-DiMeIgQx: 0.71 ± 0.10	

(*Continued*)

Table 5–(*Continued*)

Sample	Method	Units	PhIP	IQ	MeIQ	IQx	8-MeIQx	7,8-DiMeIQx	4,8-DiMeIQx	Others	Ref.
Ground beef patties, fried, 189 °C/15 min, well done			2.70 ± 0.10	0.10 ± 0.00		0.40 ± 0.00	3.00 ± 0.20		0.60 ± 0.00	IFP: 0.60 ± 0.00 IQ[4,5-*b*]: 0.40 ± 0.30 IgQx: 1.50 ± 0.30 7-MeIgQx: 9.50 ± 1.50 6,7-DiMeIgQx: 0.30 ± 0.10 7,9-DiMeIgQx: 2.20 ± 0.10	
Ground beef patties, fried, 191 °C/20 min, very well done			2.90 ± 0.10	0.20 ± 0.20		0.40 ± 0.00	3.70 ± 0.30		0.70 ± 0.00	IFP: 0.70 ± 0.00 IQ[4,5-*b*]: 0.30 ± 0.10 IgQx: 1.80 ± 0.10 7-MeIgQx: 11.70 ± 0.40 6,7-DiMeIgQx: 0.40 ± 0.10 7,9-DiMeIgQx: 3.00 ± 0.20	
Ground beef patties, 150 to 230 °C/4 to 20 min	HPLC-FD-DAD	ng/g	nd to 32	nd to 07			nd to 7.3		nd to 1.6		(Knize and others 1994c)
Ground beef patties, 160 to 250 °C, single turn	HPLC-UV/FD	ng/g	0.3 to 8.92				0.69 to 2.71		0.29 to 1.45	IFP: <0.1 to 3.97	(Salmon and others 2000)
Ground beef patties, 160 to 250 °C, multiple turns			<0.1 to 1.80				0.14 to 1.55		<0.1 to 0.45	IFP: <0.1 to 0.46	
Ground beef patties, 200 °C/12 min	HPLC-DAD	ng/g	7.83 ± 0.78				7.80 ± 0.84		2.69 ± 0.44		(Cheng and others 2007)
Ground beef patties, 200 °C/12 min, naringenin added			2.05 ± 0.18				2.58 ± 0.22		1.00 ± 0.16		
Ground beef patties, 200 °C/12 min, theaflavin-3,3′-digallate added			3.82 ± 0.87				3.58 ± 0.32		1.34 ± 0.18		
Ground beef patties, 200 °C/12 min, epicatechin gallate (ECG) added			5.50 ± 0.99				4.32 ± 0.95		1.51 ± 0.36		
Ground beef patties, 200 °C/12 min, rosmarinic acid added			5.21 ± 0.71				4.03 ± 0.88		1.46 ± 0.38		
Ground beef patties, 200 °C/12 min, carnosic acid			6.39 ± 0.40				5.72 ± 1.30		1.81 ± 0.41		
Ground beef patties, 175 to 225 °C/12 min	HPLC-FD-DAD	ng/g	0.9 to 13.3	0.7 to 2.8	0.1 to 2.0		0.5 to 3.5		0.8 to 3.0		(Balogh and others 2000)
Ground beef patties, 175 to 225 °C/20 min			6.2 to 31.4	1.3 to 5.3	0.3 to 3.5		0.8 to 5.8		0.9 to 4.8		
Ground beef patties, 225 °C/20 min oleoresin rosemary added			17.4 ± 2.9	1.5 ± 0.6	0.7 ± 0.7		3.8 ± 0.7		1.1 ± 0.6		
Ground beef patties, 225 °C/20 min, vitamin E added			9.6 ± 5.3	0.7 ± 0.2	0.8 ± 0.4		2.9 ± 1.9		1.0 ± 0.3		
Ground beef patties, 225 °C/20 min, vitamin E (surface application)			14.1 ± 6.0	6.4 ± 5.8	3.0 ± 3.0		3.9 ± 1.2		1.8 ± 0.7		
Ground beef patties, 225 °C/20 min	HPLC-FD-DAD	ng/g	17.4 ± 1.5				5.7 ± 0.5		3.1 ± 0.3		(Shin and others 2003a)
Ground beef patties, 225 °C/20 min, inuline added			8.6 ± 0.5				2.3 ± 0.4		1.2 ± 0.3		
Ground beef patties, 225 °C/20 min, oligosaccharides added			9.2 to 9.7				2.6 to 2.8		1.4 to 1.7		
Ground beef patties, 230 °C/5 min, marinated with onion, garlic and lemon juice	HPLC-UV	ng/g	nd to 0.09				0.38 to 1.22		nd to 0.45		(Gibis 2007)
Meat sauce, fried, 150 to 225 °C/6 min	HPLC-UV/FD	ng/g fresh wt	0.07 to 2.1				<2 ng to 1.1		<4 ng to 0.4		(Skog and others 1997)

Table 6–Content of pyrolytic HAAs in hamburgers.

Sample	Method	Units	Glu-P-2	Glu-P-1	Harman	Norharman	AαC	MeAαC	Trp-P-2	Trp-P-1	Ref.
100°C/10 to 17 min	HPLC-FD-DAD	ng/g					<0.1				(Knize and others 1996, 1997b)
175 to 200 °C/11.2 min	LC-ESI-MS-MS-MRM	ng/g	<0.1	<0.1	1.9 ± 0.6	0.8 ± 0.1	<0.04	<0.1	<0.1	<0.05	(Busquets and others 2004)
Very well done	HPLC-FD	ng/g					0.4	<0.02	0.3	0.03	(Ristic and others 2004)
Fried, 200 °C/10 min; fresh virgin olive oil	HPLC-FD-DAD	ng/g			2.6 to 15.0	1.4 to 4.9	nd	nd	nd	nd	(Persson and others 2003a)
"; stored (1 y) virgin olive oil					1.5 to 3.8	1.0 to 2.2	nd	nd	nd	nd	
Fried with different fats, 200 °C/8 min	HPLC-FD-DAD	ng/g	nd	nd	0.1 to 0.7	0.5 to 3.2					(Johansson and others 1995b)
Pan-fried	HPLC-FD-DAD	ng/g			7.0	12.0					(Totsuka and others 1999)
Grilled					3.63	5.98					
Fried, homemade	HPLC-ESI-MS-SIM	ng/g			nd	nd	nd	nd	0.21	0.29	(Borgen and Skog 2004)
Fried, medium done, industrial							nd	nd	0.16	0.09	
Fried, medium or well done, fast food					nd to 0.02	nd to 0.52	nd to 0.09	nd to 0.05	0.04 to 0.35	nd to 0.62	
Grilled, medium-done, fast food					0.01	0.13	0.02	0.02	0.13	nd	
Microwaved	LC-APCI-MS-MS	ng/g					nd				(Holder and others 1997)
Fast food	LC-ESI-IT-MS-SIM	ng/g			nd	nd	nd	nd	0.03	0.01	(Bang and others 2002)
Restaurant					0.1	0.08	0.02	nd	nd	nd	
Meat patty, fried, medium done, industrial	HPLC-ESI-MS-SIM	ng/g					nd	0.02	0.10	0.11	(Borgen and Skog 2004)
Meat roll, fried, well done, industrial							nd	nd	nd	nd	

cooking times and high temperature (Gross and Grüter 1992; Bordas and others 2004). Differences were found in a commercial meat flavor model system in dry and wet conditions (Bordas and others 2004). In the dry model system, cooking temperature and time influenced the formation of HAAs, although not in the linear mode. However, in wet conditions, a decrease in the formation of HAAs was detected after a long time and high temperature were applied (Bordas and others 2004).

When heat transfer is very efficient, the formation of HAAs begins immediately upon heating at around 200 °C, depending of the HAA, and within only 30 s surprisingly large amounts have been shown to be formed (Arvidsson and others 1997, 1999). Some authors found that after some further time, depending on temperature, a maximum level is reached after which the concentration more or less levels out to a plateau, except at 225 °C. The plateau is only reached for 4,8-DiMeIQx, while the amounts of 8-MeIQx and 7,8-DiMeIQx decrease after peaking, and no significant amount of PhIP is detected. These results indicate that the HAAs are not only formed, but also undergo some kind of degradation. Such degradation might have occurred at all temperatures used, but it is significant only above 200 °C for the IQx derivatives and PhIP (Knize and others 1994c; Jackson and Hargraves 1995). These results are in accordance with other studies (Knize and others 1994c; Jackson and Hargraves 1995). Thus, the formation of 8-MeIQx and 4,8-DiMeIQx in a model system containing glucose, creatine, and Thr was studied (Jackson and Hargraves 1995). In the model system, the formation of HAAs peaked after 50 to 80 min of heating at 225 and 250 °C, after that, degradation became obvious (Jackson and Hargraves 1995). The HAAs are not very stable at 225 °C, and the rate of breakdown seems to vary slightly with pH. After 30 min heating at pH 6 or 8, about 25% to 50% of the HAAs are destroyed depending on the specific compound. PhIP is the less stable followed by 7,8-DiMeIQx, 8-MeIQx, 4,8-DiMeIQx, and IQx (Arvidsson and others 1997).

However, during the first 25 min, while temperatures rase from 25 to 150 °C in fried beef, PhIP was formed predominantly, whereas MeIQ did not increase (Murkovic and Pfannhauser 2000). Even at high temperatures, MeIQ was formed to a comparable low amount. Other HAAs (8-MeIQx, IQ, 4,8-DiMeIQx, PhIP) increased significantly especially at the highest temperatures, with 8-MeIQx having the highest concentration (Murkovic and Pfannhauser 2000). The highest amount of 8-MeIQx was reached in beef at 220 °C/20 min, but no formation of 4,8-DiMeIQx was observed at 220 °C/5 min (Ahn and Grun 2005a). A longer time and higher temperatures were needed to produce the initial 20% of the formed PhIP compared with 8-MeIQx in fried beef patties (Knize and others 1994c; Balogh and others 2000). Similar results were detected by other authors (Skog and others 1997) who, surprisingly, found Trp-P-1 and Trp-P-2 in most meats and fishes fried at 225 °C, and in meat sauce prepared at 175 to 225 °C. Harman and norharman could not be quantified, although they were present in most dishes. Due to coeluting, impurities AαC and MeAαC were not properly identified (Skog and others 1997).

A higher fat content in meat results in a shorter time needed to reach a fixed meat surface temperature (Abdulkarim and Smith 1998). This is because fat is an effective heat transfer agent. Higher HAA levels are detected in cooked foods with lower fat content. The effect of fat is more pronounced on the surface where occurs more fat melting due to the higher exposure to heat (Abdulkarim and Smith 1998). As previously commented, the formation of HAAs is highly dependent on time and temperature of cooking. When the food reaches more rapidly the temperature of cooking,

Table 7–Content of thermic HAAs in hamburgers.

Sample	Method	Units	PhIP	IQ	MeIQ	IQx	8-MeIQx	7,8-DiMeIQx	4,8-DiMeIQx	Others	Ref.
Fried	HPLC-UV	ng/g fresh wt	<0.1 to 1.5	<0.1	<0.1		<0.1 to 1.3	<0.1	<0.1 to 0.3		(Zimmerli and others 2001)
Fried, homemade	HPLC-ESI-MS-SIM	ng/g	0.43				0.17				(Borgen and Skog 2004)
Fried, medium/well done, industrial			0.02/0.05			<0.01/0.14	<0.01	0.01/0.04	<0.01/0.05		
–	HPLC-ESI-MS-MS-SRM	ng/g	0.4	<0.1	<0.1		0.4	0.1			(Richling and others 1998)
100 °C/10 to 17 min	HPLC-FD-DAD	ng/g	11	nd			0.89		<0.1		(Knize and others 1997b)
Fried, 175 to 200 °C/11.2 min	LC-ESI-MS-MS-MRM	ng/g	0.6 ± 0.02	<0.04	<0.04		0.7 ± 0.1	<0.04	<0.1	DMIP: <0.2	(Busquets and others 2004)
Fried, 180 to 200 °C/12 min	GC-MS-SIM	ng/g	4.0 ± 2.6				3.5 ± 0.9		0.3 ± 0.1		(Reistad and others 1997)
Fried, 150 to 225 °C/5 to 7 min	HPLC-FD-DAD	ng/g	0.01 to 1.1	nd			nd to 2.2		nd –to 0.8		(Skog and others 1995)
Fried, 150 to 225 °C/5 to 7 min, pan residue			0.08 to 11.2	nd			0.06 to 5.8		0.02 to 1.1		
Fried, 180 to 200 °C/11 min	LC-ESI-IT-MS-MS	ng/g	0.6							4′-OH-PhIP: <0.08	(Busquets and others 2007)
Fried, 180 to 190 °C/12 min	HPLC-UV	ng/g	1.2	nd to 0.1	nd		0.03 to 2.8		nd to 0.7		(Johansson and Jägerstad 1994)
Fried, 180 to 190 °C/12 min, pan residue			nd	nd to 1.5	1.7		0.6 to 5.3		nd to 1.8		
Barbecued, 20 min			nd	nd	nd		1.0		0.2		
Commercial			nd	0.1	0.4		0.4		0.1		
Pan-fried, well done	HPLC	ng/g	0.47				0.65		0.18	IFP: 0.16	(Keating and others 2000)
Pan-fried, very well done			2.04				1.88		0.61	IFP: 0.74	
Very well done	HPLC-FD	ng/g	2.0								(Ristic and others 2004)
Griddle fried	HPLC-FD-DAD	ng/g	1.9 to 4.4	<0.1	<0.1		1.3 to 1.8		<0.1		(Knize and others 1998)
Charbroiled			1.8 to 18.4	<0.1	<0.1		0.2 to 1.8		0.1 ± 0.05		
Pan-fried, well done	HPLC-FD-DAD	ng/g	67.5				16.4		4.5		(Knize and others 1997a)
Grilled, well done			11				0.89		<0.1		
Fried, 180 to 191 °C/6 to 20 min	HPLC-FD-DAD	ng/g	<0.2 to 2.3	<0.2	<0.2		0.34 to 4.3		<0.2		(Sinha and others 1998b)
Broiled, 175 to 186 °C/6 to 12 min			<0.2	<0.2	<0.2		<0.2 to 1.6		<0.2		
Grilled/barbecued, 185 to 240 °C/9 to 38 min			<0.2 to 16.8	<0.2	<0.2		<0.2 to 4.6		<0.2		
Fried, 190 to 200 °C/24 min, very well done	HPLC-DAD	ng/g	10.2	6.8	12.6		18.3		29.5		(Warzecha and others 2004)
Fried, 198 to 277 °C/12 min	HPLC-DAD	ng/g	4.9 to 68	nd			4.3 to 16		1.3 to 4.5		(Thiebaud and others 1995)
Fried, 200 °C/10 min; fresh virgin olive oil	HPLC-FD-DAD	ng/g	4.5 to 15.8	nd	nd		1.0 to 7.7	nd	0.1 to 0.7		(Persson and others 2003a)
"; stored (1 y) virgin olive oil			2.1 to 28.0	nd	nd		1.1 to 11.7	nd	nd to 2.4		
Fried with margarine, 165 to 200 °C/8 min	HPLC-FD-DAD	ng/g	0.08 to 0.5				0.2 to 1.0		<0.02 to 0.4		(Johansson and others 1995b)
Fried with margarine, 165 to 200 °C/8 min, pan residue			0.4 to 4.6 ± 0.9	nd	nd		0.8 to 2.7 ± 0.3		0.4 to 1.3 ± 0.1		
Fried with margarine fat phase, 200 °C/8 min			1.5 ± 0.6				1.2 ± 0.1		0.2		
Fried with margarine fat phase, 200 °C/8 min, pan residue			13.3 ± 3.6	nd	nd		3.5 ± 0.3		1.0		
Fried with liquid margarine, 200 °C/8 min			1.0				1.1 ± 0.1		0.4		
Fried with liquid margarine, 200 °C/8 min, pan residue			9.7 ± 2.3	nd	nd		4.3 ± 0.5		1.1 ± 0.2		
Fried with butter, 200 °C/8 min			1.0				1.2 ± 0.2		0.2		
Fried with butter, 200 °C/8 min, pan residue			11.7 ± 3.4	nd	nd		3.2 ± 0.9		1.0		
Fried with rapeseed oil, 200 °C/8 min			1.1 ± 0.2				1.6 ± 0.1		0.4		
Fried with rapeseed oil, 200 °C/8 min, pan residue			11.4 ± 3.6	nd	nd		2.8 ± 0.2		0.8		
Fried with sunflower seed oil, 200 °C/8 min			0.9				1.2 ± 0.2		0.2		
Fried with sunflower seed oil, 200 °C/8 min, pan residue			2.0 ± 0.5	nd	nd		2.1 ± 0.4		0.5		
Ground beef patties, fried, 225 °C/20 min	HPLC-FD-DAD	ng/g	17.4 ± 1.5				5.7 ± 0.5		3.1 ± 0.3		(Shin and others 2003a)
Ground beef patties, fried with oligosaccharides or inuline, 225 °C/20 min			9.2 to 8.6				2.8 to 2.3		1.7 to 1.2		
Microwaved	LC-APCI-MS-MS	ng/g	nd	nd			nd				(Holder and others 1997)
Charcoal-grilled	HPLC-FD-DAD	ng/g	290				<0.1		0.3		(Knize and others 1996)

(*Continued*)

Table 7–(*Continued*)

Sample	Method	Units	PhIP	IQ	MeIQ	IQx	8-MeIQx	7,8-DiMeIQx	4,8-DiMeIQx	Others	Ref.
Grilled, internal temperature 65 °C;	ESI-IT-MS-MRM	Ratio signal	38.74		9.01		29.51				(Friedman and others 2009)
"; plus carvacrol			23.22 ± 1.42		6.57 ± 0.56		17.65 ± 1.55				
Grilled, internal temperature 70 °C;			131.63		21.07		84.34				
"; plus carvacrol			28.76 ± 1.94		8.76 ± 0.82		23.72 ± 2.98				
Grilled, internal temperature 80 °C;			197.02		27.02		126.83				
"; plus carvacrol			95.61 ± 6.30		23.38 ± 4.51		84.06 ± 7.73				
Grilled, medium-done, fast food	HPLC-ESI-MS-SIM	ng/g	0.06			0.02	0.02	0.02	0.03		(Borgen and Skog 2004)
Fried, medium or well done, fast food			nq to 0.04			0.05 to 0.14	0.02 to 0.18	nd	nd to 0.01		
Very well done (fast food)	HPLC-coulometric electrode array detect.	ng/g		<1.4	1.6		0.4		<2.5	DMIP: <0.8	(Gerbl and others 2004)
Commercial (fast food)	HPLC-FD-DAD	ng/g	0.1 to 0.6				<0.1 to 0.3		<0.1 to 0.1		(Knize and others 1995)
Fast food	LC-ESI-IT-MS-SIM	ng/g	0.02	nd	nd	nd	nd	0.02	0.05	DMIP: nd	(Bang and others 2002)
Restaurant			0.1	0.01	nd	nd	0.03	nd	0.04	DMIP: nd	
Meat patty, fried, medium done, industrial	HPLC-ESI-MS-SIM	ng/g	0.03				nd				(Borgen and Skog 2004)
Meat roll, fried, well done, industrial			nd				nd				

Table 8–Content of pyrolytic HAAs in bacon, sausages, and black puddings.

Sample	Method	Units	Glu-P-2	Glu-P-1	Harman	Norharman	AαC	MeAαC	Trp-P-2	Trp-P-1	Ref.
Bacon, microwaved, 600 W/3 min	HPLC-FD-DAD, HPLC-TS-MS-SIM	ng/g			nd	3.3	0.1				(Gross and others 1993)
Bacon, grilled							<1				
Bacon, fried, 170 °C/12 to 16 min					nd to 22	nd –to 30	<0.5				
Bacon, gridled	HPLC-MS	ng/g					nq				(Back and others 2009)
Bacon, oven-broiled	HPLC-FD-DAD	ng/g			32.5	59.6					(Totsuka and others 1999)
Bacon, pan-fried					5.50	40.2					
Bacon, pan-fried, 176 °C/16.1 min, very well done	LC-ESI-MS-MS-SRM	ng/g					<0.03	<0.03			(Ni and others 2008)
Bacon, oven-broiled, 175 °C/7.2 min, very well done							0.26 ± 0.04	<0.03			
Bacon, fried	GC-EI-MS-SIM	ng/g				6.7 ± 2.7	<0.05	<0.07	<0.29	0.6 ± 0.3	(Casal and others 2004)
Sausages, fried, 175 to 200 °C/9 min	LC-ESI-MS-MS-MRM	ng/g	<0.1	<0.1	0.3 ± 0.04	0.3 ± 0.05	<0.02	<0.02	<0.02	<0.02	(Busquets and others 2004)
Pork sausages patties, fried, 179 °C/21 min, very well done	LC-ESI-MS-MS-SRM	ng/g					<0.03	<0.03			(Ni and others 2008)
Pork sausages, fried, 150 to 230 °C/6 to 15 min	HPLC-FD-DAD	ng/g			nd to 0.84	nd to 3.08			nd to 0.22		(Abdulkarim and Smith 1998)
Crusts of pork sausages, fried, 150 to 230 °C/6 to 15 min					nd to 2.51	nd to 10.6			nd to 1.23		
Pork sausages, barbecued, 200 °C/12 min or 240 °C/7 min					0.10 to 1.16	0.57 to 4.20			0.03 to 0.13		
Crusts of pork sausages, barbecued, 200 °C/12 min or 240 °C/7 min					0.56 to 2.53	1.94 to 9.18			0.22 to 1.92		
Sausages, grilled, very well done	HPLC-FD	ng/g					<0.02	<0.02	1.2	1.2	(Ristic and others 2004)
Falun sausage, roasted, 200 °C/30 min	HPLC-UV/FD	ng/g			nq	nq			<0.3	<0.3	(Skog and others 1997)
Falun sausage, roasted, 200 °C/30 min, pan residue					nq	nq			<0.3	<0.3	
Cocktail sausage, fried, 225 °C/5 min					nq	nq			nq	0.5	
Cocktail sausage, fried, 225 °C/5 min, pan residue					nq	nq			<0.3	0.04	
Black pudding, fried, 225 °C/5 min					nq	nq			<0.3	<0.3	
Black pudding, fried, 225 °C/5 min, pan residue					nq	nq			nq	nq	

Table 9–Content of thermic HAAs in bacon, sausages, and black pudding.

Sample	Method	Units	PhIP	IQ	MeIQ	IQx	8-MeIQx	7,8-DiMeIQx	4,8-DiMeIQx	Others	Ref.
Bacon, microwaved, 600 W/3 min	HPLC-FD-DAD, HPLC-TS-MS-SIM	ng/g					0.1				(Gross and others 1993)
Bacon, grilled			<0.1 to 52				0.9 to 18		<1		
Bacon, fried, 170 °C/12 to 16 min			nd to 52				0.9 to 27		<0.5 to 2.4		
Bacon, pan-fried, 176 °C/16.1 min, very well done	LC-ESI-MS-MS-SRM	ng/g	4.90 ± 0.81	<0.03		0.49 ± 0.04	3.00 ± 0.21		0.72 ± 0.03	IFP: 0.16 ± 0.02 IQ[4,5-*b*]: 0.05 ± 0.06 IgQx: 1.05 ± 0.10 7-MeIgQx: 3.46 ± 0.38 6,7-DiMeIgQx: 0.08 ± 0.01 7,9-DiMeIgQx: 0.43 ± 0.05	(Ni and others 2008)
Bacon, oven-broiled, 175 °C/7.2 min, very well done			15.91 ± 0.78	<0.03		0.68 ± 0.13	2.61 ± 0.06		0.52 ± 0.02	IFP: 0.41 ± 0.04 IQ[4,5-*b*]: 0.11 ± 0.02 IgQx: 2.98 ± 0.37 7-MeIgQx: 9.08 ± 0.37 6,7-DiMeIgQx: 0.24 ± 0.01 7,9-DiMeIgQx: 0.77 ± 0.11	
Bacon, microwaved, 1.8 to 3.3 min	HPLC-FD-DAD	ng/g	<0.2 to 3.1	<0.2	<0.2		<0.2 to 1.5		<0.2		(Sinha and others 1998a)
Bacon, oven-broiled, 175 to 185 °C/4 to 7 min			1.4 to 30.3	<0.2	<0.2		<0.2 to 4		<0.2		
Bacon, fried, 176v to 177 °C/4 to 16 min			<0.2 to 4.8	<0.2	<0.2		0.4 to 4.3		<0.2		
Fat from bacon, fried, 176 to 177 °C/4 to 16 min			<0.2 to 2.3	<0.2	<0.2		<0.2 to 0.6		<0.2		
Bacon, grilled, well done	HPLC-FD-DAD	ng/g	<0.1 to 36				1.0 to 27		<0.1 to 9.3		(Knize and others 1997a)
Bacon, grilled, 230 °C/1.5 min	LC-APCI-MS-MS-SRM	ng/g	4.97	0.42			1.61	0.05	0.94		(Guy and others 2000)
Bacon, pan-fried, 230 °C/1.5 min			28.4	0.53			8.14	0.41	4.51		
Bacon, fried, 225 °C/12 min	GC-NICI-MS	ng/g	1.6 to 2.7				0.9 to 1.2		0.2 to 0.3		(Murray and others 1993)
Bacon, fried, 208 °C/12 min	HPLC-DAD	ng/g	106	nd			45		12		(Thiebaud and others 1995)
Bacon, fried, 150 to 225 °C/4 to 8 min	HPLC-FD-DAD	ng/g	0.3 to 4.5	nd			nd to 23.7		nd to 1.4		(Skog and others 1995)
Bacon, fried, 150 to 225 °C/4 to 8 min, pan residue			0.06 to 0.8	nd			nd to 23.3		nd		
Bacon, fried, 150 °C/5 to 10 min	HPLC-UV	ng/g	0.2 to 1.0	3.8 to 10.5	nd to 1.7		2.5 to 2.8		1.0 to 3.4		(Johansson and Jägerstad 1994)
Bacon, fried, 150 °C/5 to 10 min, pan residue			nd	nd	nd		0.2 to 5.9		0.2 to 1.7		
Bacon, fried	GC-EI-MS-SIM	ng/g	36.4 ± 11.3	1.6 ± 0.1	2.8 ± 0.8	<0.05	<0.05	<0.06	1.1 ± 0.4		(Casal and others 2004)
Bacon, griddled	HPLC-MS	ng/g	168.2	nq				nq	nq	TriMeIQx: 79.9	(Back and others 2009)
Luncheon meat	HPLC-FD-DAD	ng/g	0.21				0.10	<0.1	<0.1	IFP: nd	(Wong and others 2005)
Sausages, fried, 160 °C/6 min	HPLC-UV	ng/g	0.1	0.1	0.2		0.7		0.2		(Johansson and Jägerstad 1994)
Sausages, fried, 160 °C/6 min, pan residue			nd	0.7	1.0		2.5		0.2		

(*Continued*)

Table 9–(*Continued*)

Sample	Method	Units	PhIP	IQ	MeIQ	IQx	8-MeIQx	7,8-DiMeIQx	4,8-DiMeIQx	Others	Ref.
Sausage links, pan-fried, 175 to 177 °C/9 to 21 min	HPLC-FD-DAD	ng/g	<0.2 to 0.1	<0.2	<0.2		<0.2 to 1.3		<0.2		(Sinha and others 1998a)
Sausage patties, pan-fried, 175 to 179 °C/8 to 21 min			<0.2	<0.2	<0.2		<0.2 to 5.4		<0.2		
Pork sausages patties, fried, 179 °C/21 min, very well done	LC-ESI-MS-MS-SRM	ng/g	0.23 ± 0.02	0.07 ± 0.00		0.72 ± 0.04	5.07 ± 0.01		0.72 ± 0.03	IFP: 0.32 ± 0.02 IQ[4,5-*b*]: 0.10 ± 0.02 IgQx: 1.80 ± 0.11 7-MeIgQx: 8.37 ± 0.38 6,7-DiMeIgQx: 0.17 ± 0.01 7,9-DiMeIgQx: 1.25 ± 0.05	(Ni and others 2008)
Sausages, fried, 175 to 200 °C/9 min	LC-ESI-MS-MS-MRM	ng/g	<0.2	<0.04	<0.4		<0.1	<0.1	<0.5	DMIP: <0.5	(Busquets and others 2004)
Pork sausages, fried, 180 to 200 °C/9 min	HPLC-ESI-IT-MS-MS	ng/g	<0.1							4′-OH-PhIP: 0.5	(Busquets and others 2007)
Pork sausages, fried, 150 to 230 °C/6 to 15 min	HPLC-FD-DAD	ng/g	nd to 1.13				nd to 0.72				(Abdulkarim and Smith 1998)
Crusts of pork sausages, fried, 150 to 230 °C/6 to 15 min			nd to 5.83				nd to 3.44				
Pork sausages, barbecued, 200 °C/12 min or 240 °C/7 min			0.09 to 1.27				0.35 to 0.80				
Crusts of pork sausages, barbecued, 200 °C/12 min or 240 °C/7 min			0.09 to 2.35				nd to 1.97				
Sausages, grilled, very well done	HPLC-FD	ng/g	0.8								(Ristic and others 2004)
Sausages, grilled, very well done (fast food)	HPLC-coulometric electrode array detector	ng/g		5.1	<2.1		<1.5		<2.5	DMIP: 1.5	(Gerbl and others 2004)
Falun sausage, fried, 150 to 225 °C/4 min	HPLC-FD-DAD	ng/g	nd to 0.1	nd			nd		nd to 0.07		(Skog and others 1995)
Falun sausage, fried, 150 to 225 °C/4 min, pan residue			0.06 to 0.4	0.05 to 0.2			0.03 to 0.2		0.04 to 0.1		
Falun sausage, fried, 160 °C/5 min	HPLC-UV	ng/g	nd	0.3	nd		0.6		nd		(Johansson and Jägerstad 1994)
Falun sausage, fried, 160 °C/5 min, pan residue			4.5	1.6	2.3		7.3		2.8		
Falun sausage, roasted, 200 °C/30 min	HPLC-UV/FD	ng/g	<0.4 ng				<2 ng		<4 ng		(Skog and others 1997)
Falun sausage, roasted, 200 °C/30 min, pan residue			<0.4 ng				<0.01		<0.01		
Cocktail sausage, fried, 150 to 225 °C/5 min			0.02 to 0.1				<2 ng to 0.1		<4 ng		
Cocktail sausage, fried, 150 to 225 °C/5 min, pan residue			<0.01 to 0.02				<2 ng to 0.02		<4 ng		
Black pudding, fried, 150 °C/8 min	HPLC-UV	ng/g	nd	0.2	nd		0.5		0.5		(Johansson and Jägerstad 1994)
Black pudding, fried, 150 °C/8 min, pan residue			nd	nd	0.3		0.05		0.03		
Black pudding, fried, 150 to 225 °C/5 min	HPLC-UV/FD	ng/g	0.06 to 0.2				<2 ng to 0.9		<4 ng		(Skog and others 1997)
Black pudding, fried,150 to 225 °C/5 min, pan residue			<0.01				<2 ng		<4 ng		

(*Continued*)

Table 9–(*Continued*)

Sample	Method	Units	PhIP	IQ	MeIQ	IQx	8-MeIQx	7,8-DiMeIQx	4,8-DiMeIQx	Others	Ref.
Hot-dogs, fried, 176 °C/4 to 18 min, or oven-broiled, 180 to 185 °C/3 to 10 min, or grilled/barbecued, 232 to 260 °C/5 to 15 min, or boiled, 100 °C/5 min	HPLC-FD-DAD	ng/g	nd	nd	nd		nd		nd		(Sinha and others 1998a)
Ham slices, pan-fried, 175 to 176 °C/5 to 19 min			<0.2 to 0.3	<0.2	<0.2		<0.2 to 1.8		<0.2		
Ham, grilled, well done	HPLC-FD-DAD	ng/g	1.5				2.3		0.2		(Knize and others 1997a)

the heat exposure period is shorter. Therefore, although the content of fat is lower, the formation of HAAs is higher. For example, flame grilling produced AαC in beef grilled over open flames, but not in the chicken under these conditions. AαC was formed by pyrolysis of meat juices dripping into the flames, and it is carried with smoke and deposited on the meat surface. Thus, beef with 15% of fat contains more AαC than beef with 30% of fat (Knize and others 1997b).

Effect of cooking method. The cooking method has a considerable influence on the formation of mutagenic activity (Wu and others 1997; Sinha and others 1998a, 1998b; Balbi and others 2001; Skog and others 2003; Sanz Alaejos and others 2008b). The meat type and the ingredients added also affect the formation of HAAs. For example, hamburgers cooked in convection ovens, on a contact fryer or at low temperatures in a deep fat fryer did not exhibit any mutagenic activity. Some of the hamburger ingredients can inhibit the formation of HAAs. Divergent results can be due to several causes, one of them is the fat content, although the role of fat in the HAAs formation is unclear (Skog and others 2003).

Microwave cooking is a mild type of treatment, because the heat is generated inside the product and the surface temperature does not increase above the temperature of the other parts of the food. However, it has been found to cause the formation of carboline types of HAAs, while frying causes the formation of both IQ and carboline types of HAAs (Chiu and others 1998). Pretreatment with microwaves before cooking lowered the formation of some IQ and IQx types because the fat, water, and HAA precursors are reduced (Felton and others 1992, 1994).

Cold cuts and precooked meat samples (ham, summer sausage, and bologna) do not contain detectable levels of HAAs. This is due to the low temperature of cooking during the processing/cooking of these products (Stavric and others 1996; Abdulkarim and Smith 1998).

Preparation methods such as boiling, oven roasting, and deep-frying are generally "milder" and much lower amounts of HAAs are formed (Zimmerli and others 2001).

In general, the 3 high-temperature cooking methods (pan-frying, grilling/barbecuing, and oven-broiling) seem to cause the highest HAA concentrations, especially of PhIP (Zimmerli and others 2001; Sinha 2002; Keating and Bogen 2004). During cooking in a deep fat fryer, a significant increase in mutagenic activity with increasing oil temperature was found (Skog and others 2003). Pan-frying and grilling/barbecuing appear to produce more 8-MeIQx and PhIP. The reason may be the shorter length of time needed for the meat to reach the same internal temperature or some other cooking-related factor. The parameters that appear to be important in forming HAAs are (1) direct contact of the meat with a hot flat surface such as in pan-frying, or (2) very high temperature such as in grilling/barbecuing (Sinha and others 1998b). Another reason that has been pointed out is that during frying, baking/roasting, or grilling/barbecuing water is generally lost from the food item, resulting in decreasing water content from the inner to the outer parts, and in the formation of a relatively dry surface. In that surface or in its proximity, where the temperature is higher, the HAAs precursors are concentrated and they can react to form the HAAs. These physical and chemical changes affect the mass and heat transport (Skog and others 2000). Similarly, it occurs during the smoking process for food conservation.

"Provola" cheese samples from Calabria (Italy), smoked using traditional methods, and using commercial buffered smoke were studied to evaluate the residual levels of HAA (Naccari and others 2009). The traditional process of smoking is carried out in uncontrolled conditions with regard to temperature, humidity, type of wood for combustion, which can have an influence in the production of HAAs and, besides, the smoke generated comes into direct contact with the cheese. In European countries, commercial buffered smoke is now used for smoking foods. The commercial buffered smoke is produced by the combustion (pyrolysis) of wood with restricted availability of oxygen. The liquid smoke flavoring is obtained by fractionating the condensate. The use of commercial buffered smoke has several advantages over traditional smoking techniques, such as easy, rapidity, and cleanliness of application, uniformity of the product, reproducibility of the characteristics obtained in the end product (buffered smoked samples), and controllability of the amount of toxic compounds in smoke flavorings before their addition to foods (Simon and others 2005). The results show the presence of 6 HAAs in all parts of "Provola" cheese naturally smoked by traditional methods. The rind is the most contaminated part and the core the least. No residual levels of HAAs were found in buffered smoked samples (Naccari and others 2009).

For the same level of doneness, different types of fish or meat (chicken, bacon, beef, beef steak) contain different levels of HAAs (Sinha and others 1996; Sinha and Rothman 1997; Sinha and others 1998b; Thomson 1999; Costa and others 2009). But the way that each of these meat items is cooked affects the production of HAAs in a major way. Oven-broiled very well done steaks and grilled/barbecued steaks differ widely in content of HAAs (Sinha and others 1996; Sinha and Rothman 1997; Sinha and others 1998b). Even within the same type of meat and doneness level, place of cooking (restaurant, fast food restaurant, and home), or cooking method can play a major role in the content of HAAs (Sinha and others 1996; Sinha and Rothman 1997; Sinha and others 1998b). Some fast food meat (Olsson and others 2002) and

offal products (Khan and others 2009) appear to contain lower amounts of HAAs. Harman and norharman are the most abundant HAAs in some offal products cooked by stir-frying, with or without using cooking additives (Khan and others 2009). In this cooking method, the heat transfer is reduced because of the frequent stirring.

The frequency of turning of the meat during cooking, a single turn or multiple turns, has been studied (Salmon and others 2000). Turning the sample every minute, while cooking, results in lower average amounts of total HAAs compared with meats turned just once. While 8-MeIQx was formed in all the samples turned every minute, it was present at lower levels compared with the patties turned just once during cooking. In several cases, frequent turning of the meat prevented detectable levels of 4,8-DiMeIQx, PhIP, and IFP from forming, whereas the 4 studied HAAs were formed for meats turned only once, except for IFP. The upper surface of the meat loses heat to the air because of convection and evaporation of water from the surface. Longer cooking times are associated with higher levels of HAAs. On the other hand, HAA precursors in the meat might not move from the center of the patty toward the hot cooking surface before the sample is turned over. Hence, some of the precursors would be less likely to reach the lower meat surface and become hot enough to begin forming HAAs before the meat is turned and the fluid flow reverses direction. The created conditions are less favorable for formation of HAAs and meat cooks better with more frequent turning (Salmon and others 2000). Adding water-binding compounds, such as salts, starch, soy protein, and so on, can restrict the transport of precursors (Skog and others 1992a; Jägerstad and others 1998; Persson and others 2003b; Shin and others 2003b; Persson and others 2004; Shin and Ustunol 2004; Shin 2005).

Coating with breadcrumbs was thought to be a way of inhibiting the formation of HAAs in the food. The coating would act as an insulating layer, decreasing outer food temperature. But results are not clear when comparing with pan residues, perhaps due to the coating being very thin (Skog and others 1997). No mutagenic activity was observed in the coated hamburger samples cooked in a deep fat fryer, but this activity was high when the samples were cooked without coating (Skog and others 2003). Similar results were obtained for deep-fried chicken fillets. The coating of chicken fillets that were deep fried before cooking in a convection oven contained more HAAs than the meat (Skog and others 2003). Norharman levels were higher in beef steak and pork loin, both coated fried, when compared with fried and griddled at the same temperatures, respectively (Busquets and others 2008).

Roasting, whereby the heat is transferred to the food by air produces less HAAs than frying (Skog and others 1997) and charcoaling (Gu and others 2001) where the food is in direct contact with the heated pan or charcoal. When convection ovens are used, more HAAs are produced at a high air temperature and at a high air velocity than at a low air velocity (Skog and others 2003). The air temperature is the parameter that has more influence in the production of HAAs. The presence of steam also reduces the mutagenic activity, except when high air temperature is applied in combination with high air velocity. In this case, steam does not seem to have any pronounced effect in these ovens (Skog and others 2003).

Grilling time increases the PhIP formation in chicken breast meat, either left intact or in ground patties (Knize and others 1997a). In all the cases, either pan-fried, oven-broiled, or grilled/barbecued, PhIP was formed in higher amounts in the whole skinless, boneless chicken breast meat sample than in the ground sample (Sinha and others 1995; Knize and others 1997a). Also, 8-MeIQx and DiMeIQx were present, but no IQ or MeIQ was detected (Sinha and others 1995). The reasons for this are not clear, but the temperature at the meat surface or the availability of precursors may be affected by grinding the muscle tissue (Knize and others 1997a).

The total levels of HAAs in fresh pork sausages were increased by 70% by grilling as contrasted to frying (Abdulkarim and Smith 1998). However, bacon samples that were pan-fried on a smooth heating surface contained 5-fold higher HAA levels than samples cooked on a grill surface, even though time of cooking and temperature applied were the same for both preparations (Guy and others 2000).

In fish, pan-frying and oven cooking generated low levels of HAAs, whereas prolonged barbecuing and high temperature mainly facilitated formation of PhIP and carbolines (Gross and Grüter 1992). The barbecuing conditions influenced significantly the formation of HAAs in sardines and salmon (Costa and others 2009). For a similar medium doneness, levels of PhIP, Glu-P-1, and α-carbolines are significantly higher in salmon samples barbecued near the heat source (at 280 to 300 °C), but no significant differences were found between levels of HAAs in salmon samples barbecued at longer distance from the heat source (180 to 200 °C) using either charcoal or an electrical griddle. No HAAs were detected in the inner part of grilled fillets; only in crust and in cooked salmon fillet (Costa and others 2009).

Stewing and boiling of poultry products did not lead to the formation of detectable amounts of HAAs, probably because cooking temperature does not exceed 100 °C (Skog and Solyakov 2002). However, in one study, boiled chicken samples contained harman and norharman (Solyakov and Skog 2002). The boiling of meat products (pig, beef, chicken) for several hours to obtain a gelatinous structure of the final meat dish (aspic or galantine) is a common practice in Russia. Harman and norharman were detected both in meat and bouillon (Solyakov and Skog 2002). Foods that were steamed or poached, or smoke-dried, showed no detectable HAAs (Zimmerli and others 2001).

Marinating prior to cooking is used frequently for chicken, and usually goes before flame-grilling, a relatively high-temperature cooking practice. These high temperatures have been associated with unusually high levels of PhIP (Sinha and others 1995). Meat is marinated for a variety of reasons, including improvement of flavor, tenderness, and moistness of the cooked product. This cooking preparation greatly reduces the amount of PhIP produced during cooking, but not 8-MeIQx (Tikkanen and others 1996; Knize and others 1997b; Salmon and others 1997). Although 8-MeIQx increased at some time points, the overall effect was a decrease in the total detected HAAs (Salmon and others 1997). Nevertheless, great variations without any clear correlation with mutagenicity are observed in the amounts of HAAs between chicken samples treated with the same or different marinades (Tikkanen and others 1996; Busquets and others 2006). In addition, the variation observed in the amounts of HAAs between equivalent products makes it difficult to estimate their concentrations in foods. At 110 to 170 °C, the chicken samples showed low or no mutagenicity (Tikkanen and others 1996). On the other hand, beefsteaks marinated with teriyaki sauce or turmeric–garlic sauce in Hawaiian style had lower PhIP and lower 8-MeIQx levels than unmarinated meat, and these levels diminished with time. On the contrary, marinating with Western commercial honey barbecue sauce caused initially a slight increase in PhIP and 8-MeIQx that were decreasing with time (Nerurkar and others 1999).

"Chinese marinated" is a traditional Chinese cooking method that means immersing the food samples in a marinade for 1 h or longer at about boiling temperature. Thus, food is mixed with several kinds of ingredients, such as soy sauce, sugar, salt, spice, and seasonings (Lan and Chen 2002; Lan and others 2004; Salmon and others 2006). This definition differs from what most people understand about marinating, which implies preincubation with a fluid of some sort to impart flavor prior to cooking. For all the food samples, marinated juice contains a higher amount of HAAs than marinated food (Lan and Chen 2002; Lan and others 2004), and the content of each studied HAA increased with increasing levels of soy sauce (0% to 20%) or sugar (0% to 5%) (Lan and Chen 2002). Soy sauce plays a more important role for formation of HAAs than rock candy (also called rock sugar). The addition of glucose during marinating promotes the formation of HAAs. Buckwheat and clover honeys were chosen for their high and low antioxidant capacity, respectively (Shin and Ustunol 2004). For both types of honey, 30% honey in the marinade formulation was more effective in inhibiting formation of HAAs and overall mutagenicity, but marinades containing buckwheat honey were the most effective (Shin and Ustunol 2004).

The types of HAAs found in cooked, marinated pork were higher than those found in cooked, marinated eggs or bean cakes (Table 10, 11, 18, 19, 22, and 23). This is probably because ground pork is used as raw material for cooking and a larger surface area can be exposed when compared to both eggs and bean cakes in solid forms. Also, the higher fat content in pork may facilitate the formation of IQ-type HAAs through Maillard reaction products such as pyridine from lipid degradation products (Lan and Chen 2002). Another possible explanation is that the fat can retard the penetration of ascorbic acid from the ingredients, and thus reduce its antioxidant activity, since in the presence of α-tocopherol, the total amounts of HAAs declined in marinated pork and bean cake. The greater inhibitory effect of α-tocopherol toward HAAs formation in marinated pork could be attributed to the higher fat content when compared to eggs or bean cakes, and thus the solubility of α-tocopherol in pork is enhanced (Lan and others 2004). But a more likely reason for finding a greater variety of HAAs in cooked pork than in cooked egg or bean cake is due to the higher presence of creatin(in)e in pork. The creatin(in)e can react with Maillard products to form IQ-type HAAs. Without creatine, this class of HAAs does not form.

On the other hand, an increasing trend was observed for all the levels and total amounts of HAAs in marinated pork and juice, as well as in eggs during heating for a cooking period of 1 to 32 h. Similarly, a large increase of the total amount of HAAs in marinated juice after 32 h was detected (Lan and others 2004). However, in honey-containing marinades, increasing marinating time has no effect on formation of HAAs in cooked meats (Shin and Ustunol 2004). Since the Chinese marinated foods are often cooked at about 100 °C for an extensive period of time in the presence of juice, the degradation of HAAs would be difficult to occur unless a longer heating time was used (Lan and Chen 2002; Lan and others 2004).

Chinese people from Singapore prepare chicken and fish samples in their homes, using their usual marinade recipe and cooking technique (Salmon and others 2006). Marinating chicken reduced PhIP significantly, but the levels of the other HAAs were not significantly lower than in nonmarinated chicken. Fish contains lower levels of each HAA in relation with marinated or nonmarinated chicken. The cooking surface temperature was significantly higher for fish than for either type of chicken, and it could be expected that concentration of HAAs would be higher; however, quite the opposite was observed. It is possible that the high water content in fish could mitigate the effects of the higher surface temperature and inhibit formation of HAAs. In addition to that, the difference in HAA concentrations in cooked fish *compared with* chicken may also be due to differences in the amounts of precursors. On the other hand, the variety in marinade recipes yields great variability in terms of efficacy of HAA inhibitions or promotions (Salmon and others 2006).

Effects of the different degrees of doneness. Degree of doneness, which is often closely related to surface browning and total cooking time, is a key issue for production of HAAs in cooked meat (Sinha and others 1998b; Keating and others 2000; Gu and others 2002; Warzecha and others 2004). Although all the cooked meat has some mutagenic activity, meat cooked at ≤150 °C to rare or medium-rare doneness showed fewer mutagens at lower content than meat cooked to well done (≥150 °C) (Abdulkarim and Smith 1998; Sinha and others 1998b). The individual HAAs measured are not produced to the same extent by each cooking method and doneness level (Sinha and others 1998b). The internal part of the meat products showed lower HAA contents than the surface (Wild 1996; Abdulkarim and Smith 1998; Steinmann and Fischer 2000; Borgen and Skog 2004). The formation of the crust is the result of steady transportation of water and dissolved compounds such as amino acids and creatinine to the surface by capillary flow to the evaporation zone. Thus, the precursors of HAAs are concentrated on or near the surface of the meat where there is the highest temperature. Prolonged cooking times at the same temperature induced a marked increase in the formation of some HAAs (Gross and Grüter 1992; Steinmann and Fischer 2000; Gu and others 2002). But 8-MeIQx and PhIP showed an apparent decrease with time during pan broiling (Gross and Grüter 1992). Increases of degree of doneness caused increments in the content of PhIP and DiMeIQx (Sinha and others 1995) or of PhIP and 8-MeIQx (Gu and others 2002).

Chicken breasts pan-fried at 190 and 220 °C had similar color measurements, but the amounts of 8-MeIQx and especially PhIP differed markedly (Solyakov and Skog 2002). The results showed that it is not possible to estimate the content of HAAs only from color measurements. In numerous assessments of human exposure to HAAs, photographs showing a variety of surface color have been used to estimate the degree of doneness and indirectly the content of HAAs. Color development increases with cooking temperature, but no correlation exists with content of HAAs (Solyakov and Skog 2002). The relation between the degree of doneness and surface browning may differ because some people fry their meat at a high temperature for a short period of time to obtain a brown surface, while the interior is not cooked through. In the same way, it is possible to achieve a similar degree of browning without applying excessive temperature and thus avoiding the formation of increased amounts of HAAs (Zimmerli and others 2001). In relation to pork chops, as judged by the human eye and appearance on the photographs included in questionnaires, the crusts of the fried RN^-/rn^+ chops are considerably darker in color than those of the rn^+/rn^+ chops at each frying temperature. Thus, by choosing meat from pigs carrying the RN^- allele and by frying the meat at lower temperatures, the contribution of pork chops to the total intake of HAAs could be even further reduced (Olsson and others 2005).

Sinha and others (1998a) analyzed the content of HAAs of different pork products cooked by frying, oven-broiling, boiling, or grilling/barbecuing with different degrees of doneness. PhIP and

Table 10–Content of pyrolytic HAAs in pork.

Sample	Method	Units	Glu-P-2	Glu-P-1	Harman	Norharman	AαC	MeAαC	Trp-P-2	Trp-P-1	Ref.
–	HPLC-ESI-MS-MS-SRM	ng/g	<0.1	<0.1							(Richling and others 1998)
Heated, lyophilized, 180 to 220 °C/10 to 30 min	HPLC-API-ES-MS	ng/g					1.4 to 71	1.4 to 63	nq	9 to 190	(Messner and Murkovic 2004)
275 °C/30 min	HPLC-FD-DAD	ng/g fresh wt			16.0	58.3					(Pais and others 1999)
Longissimus dorsi muscle, grilled, 200 °C; nonaged; normal quality, internal temperature 70 °C, medium done	HPLC-API-ESI-MS-MS	ng/g			0.08 ± 0.03	0.12 ± 0.06					(Polak and others 2009b)
"; internal temperature 95 °C, well done					0.14 ± 0.05	0.35 ± 0.17					
Longissimus dorsi muscle, grilled, 200 °C; nonaged; PSE quality, internal temperature 70 °C, medium done					0.08 ± 0.02	0.13 ± 0.03					
"; internal temperature 95 °C, well done					0.14 ± 0.06	0.34 ± 0.17					
Longissimus dorsi muscle, grilled, 200 °C; 10 d aged; normal quality, internal temperature 70 °C, medium done					0.11 ± 0.03	0.30 ± 0.10					
"; internal temperature 95 °C, well done					0.18 ± 0.05	0.60 ± 0.11					
longissimus dorsi muscle, grilled, 200 °C; 10 d-aged; PSE quality, internal temperature 70 °C, medium done					0.11 ± 0.01	0.29 ± 0.06					
"; internal temperature 95 °C, well done					0.20 ± 0.03	0.70 ± 0.15					
Blackened, 100 °C/10 to 17 min	HPLC-FD-DAD	ng/g					<0.1				(Knize and others 1996, 1997b)
Fillet, fried, 225 °C/7 min, crust	GC-NIC-MS-SIM	ng/g dry wt					nq	3.2	nq	0.8	(Skog and others 1998b)
Chop, roasted, 175 °C/60 min, pan residue							0.04	0.08	<2 ng	<0.1 ng	
Chop, oven-broiled	HPLC-FD-DAD	ng/g			11.2	17.3					(Totsuka and others 1999)
Chop, pan-fried					0.62	2.39					
Chop, fried, 176 °C/15 min, very well done	LC-ESI-MS-MS-SRM	ng/g					<0.03	<0.03			(Ni and others 2008)
Chop, fried, 200 °C/6 min	HPLC-DAD	ng/g			0.4 to 1.9	1.8 to 3.6					(Olsson and others 2002)
Chop, fried, 200 °C/6 min, noncarriers of RN$^-$ allele					0.7 ± 0.5	1.9 ± 0.6					
Chop, fried, 200 °C/6 min, carriers of RN$^-$ allele					1.6 ± 0.5	3.4 ± 0.6					
Chop, fried, 160 °C/6 min, noncarriers of RN$^-$ allele	LC-ESI-IT-MS-SIM	ng/g			1.08	0.64					(Olsson and others 2005)
Chop, fried, 200 °C/6 min, noncarriers of RN$^-$ allele					0.69	1.90					
Chop, fried, 160 or 200 °C/6 min, carriers of RN$^-$ allele					nd	nd					
Knuckle, very well done	HPLC-FD	ng/g					<0.02	<0.02	0.9 to 3.1	0.9 to 1.2	(Ristic and others 2004)
Loin fatless, fried, 175 to 200 °C/10 min	LC-ESI-MS-MS-MRM	ng/g	<0.1	<0.04	1.4 ± 0.5	2.3 ± 0.2	0.2 ± 0.01	<0.1	<0.4	<0.05	(Busquets and others 2004)
Loin, coated-fried, 180 to 200 °C/7 min	HPLC-ESI-IT-MS-MS	ng/g			0.7 ± 0.3	6.7 ± 1.6	nd	nd	nd	nd	(Busquets and others 2008)
Loin, griddled, 180 to 200 °C/10 min					1.1 ± 0.3	2.2 ± 0.3	<0.1	nd	nd	nd	
Loin, griddled	HPLC-MS	ng/g			990.9	412.7	nq				(Back and others 2009)
Rib, boned, grilled, commercial	LC-ESI-MS	ng/g	nd	nd	nd to 267.0	nd to 33.2	nd	nd to 9.1	nd	nd	(Jo and others 2008)
Rinds, 100 °C/10 to 17 min	HPLC-FD-DAD	ng/g					<0.1				(Knize and others 1997b)
Raw pork, *longissimus dorsi* muscle	HPLC-UV-FD	ng/g	nd		nd	nd	nd	nd	nd		(Liao and others 2009)
Pork floss, stir fried, 100 °C			nd		11.18 ± 1.26	35.40 ± 11.54	1.00 ± 0.16	0.25 ± 0.07	nd		
Pork floss, stir fried, 125 °C			nd		11.70 ± 2.76	42.75 ± 14.83	1.97 ± 0.50	0.49 ± 0.15	nd		
Pork floss, stir fried, 150 °C			2.27 ± 1.22		24.86 ± 9.00	50.63 ± 10.47	2.58 ± 0.58	0.6 ± 0.29	5.09 ± 0.45		
Pork floss, stir fried, 125 °C, 0.1% vitamin C			–		10.35 ± 2.96	31.07 ± 9.69	2.23 ± 0.34	0.48 ± 0.11	–		
Pork floss, stir fried, 125 °C, 0.1% vitamin E			–		9.92 ± 5.69	24.01 ± 9.70	nd	nd	–		
Marinated, with 1% rock candy, without soy sauce	HPLC-DAD	ng/g					<0.21 ng			<0.12 ng	(Lan and Chen 2002)
Marinated, with 1% rock candy and 5% to 20% soy sauce							0.43 to 0.62			0.53 to 0.62	
Marinated, with 10% soy sauce, without rock candy							0.47 ± 0.26			0.14 ± 0.35	

(*Continued*)

Table 10–(*Continued*)

Sample	Method	Units	Glu-P-2	Glu-P-1	Harman	Norharman	AαC	MeAαC	Trp-P-2	Trp-P-1	Ref.
Marinated, with 10% soy sauce and 0.5% to 5% rock candy							0.58 to 0.74			0.45 to 0.63	
Marinated, with 10% soy sauce and 1% rock candy, 1 to 4 h	HPLC-DAD	ng/g					0.62 to 1.27			0.63 to 2.45	(Lan and others 2004)
Marinated, with 10% soy sauce and 1% rock candy, 8 to 32 h							1.37 to 2.46			4.34 to 8.03	
Marinated, with 10% soy sauce and 1% rock candy, 1 h							0.56 ± 0.78			0.58 ± 0.24	
Marinated, with 10% soy sauce and 1% rock candy, 1 h, with ascorbic acid							1.26 ± 1.75			0.31 ± 0.75	
Marinated, with 10% soy sauce and 1% rock candy, 1 h, with α-tocopherol							0.42 ± 1.24			0.36 ± 0.34	
Marinated, with 10% soy sauce and 1% rock candy, 1 h, with BHT							0.52 ± 1.28			0.42 ± 0.58	
Pork juice, marinated, with 1% rock candy, without soy sauce	HPLC-DAD	ng/mL					<0.21 ng			<0.12 ng	(Lan and Chen 2002)
Pork juice, marinated, with 1% rock candy and 5% to 20% soy sauce							0.77 to 1.05			0.82 to 1.36	
Pork juice, marinated, with 10% soy sauce, without rock candy							0.59 ± 0.45			0.43 ± 0.36	
Pork juice, marinated, with 10% soy sauce and 0.5% to 5% rock candy							0.87 to 1.05			0.72 to 1.02	
Pork juice, marinated, with 10% soy sauce and 1% rock candy 1 to 4 h	HPLC-DAD	ng/g					0.93 to 1.85			1.03 to 3.47	(Lan and others 2004)
Pork juice, marinated, with 10% soy sauce and 1% rock candy, 8 to 32 h							2.15 to 2.65			5.62 to 8.44	
Stewing cubes, fried, 225 °C/5 min	HPLC-UV-FD	ng/g			nq	nq			<0.3 ng	<0.3 ng	(Sinha and others 1998b)
Stewing cubes, fried, 225 °C/5 min, pan residue					nq	nq			0.1	<0.3 ng	

8-MeIQx levels varied substantially due to pork product, cooking method, and doneness level. The well and very well done pan-fried bacon showed the highest levels of PhIP, whereas the very well done pan-fried pork chops contained the highest levels of 8-MeIQx. Hot dogs had no detectable levels of these HAAs even when cooked to very well done by the usual cooking methods (Olsson and others 2002). Likewise, the same HAA contents increased in hamburgers and steaks with greater degrees of doneness mainly in pan-fried and grilled/barbecued meat (Sinha and others 1998b).

Content of HAAs in meat drippings and pan residues. Meat drippings contain, frequently, similar amounts of HAAs and similar mutagenic activity as the meat itself, and can therefore be considered to be of equal importance as a source of mutagenic compounds in diets where drippings are consumed. But, sometimes, pan residues contain more levels of HAAs (Johansson and Jägerstad 1994; Johansson and others 1995b; Skog and others 1995, 1997; Pais and others 1999; Janoszka and others 2009). Even in some foods, HAAs are detected only in the pan residues and not in the solid material; therefore, if pan residues are included, HAAs are found in all the dishes analyzed except the fried egg (Skog and others 1997). This is not surprising since egg does not contain appreciable amounts of creatine. The concentration of some HAAs formed in drippings was lower than in chicken breast meat (Skog and others 1997; Pais and others 1999; Solyakov and Skog 2002) or in cod fillet (Skog and others 1997).

Simulated meat flavors, beef extracts, and, in some countries, pan residues are used to prepare gravies. The extracts, often described as "processed food flavors" (PFFs), are normally produced commercially by exposing raw meat to prolonged heating at higher than typical cooking temperatures. Although there are PFFs produced without meat, most of them contain meat. The presence of other natural components, such as vegetables, spices, known to possess antimutagenic activity may modify the total mutagenic activity either by enhancement or by inhibition.

Also, the contribution of gravy to the total intake of HAAs can be substantial (Sinha and others 1998b; Janoszka and others 2009).

Effect of sugar. The formation of HAAs may be retarded after incorporation of excessive molar proportions of sugar versus the other precursors (Skog and others 1992a; Skog 1993; Tai and others 2001; Solyakov and Skog 2002; Bordas and others 2004). Thus, in fried fish fiber (*Trachinoephlus myops*), after adding 9% and 14% sugar, the amounts of amino acids in tissues dropped, while the HAAs increased by 85% and 15%, respectively. However, with 19% sugar, the HAAs showed a great decrease, while the amino acids showed an increase. This phenomenon may be explained by the Maillard reaction. The Maillard reaction products are more readily formed at a high sugar level than at a low sugar level. These, in turn, react with creatine or creatinine, and thus the amount of glucose diminishes greatly. At a lower level of sugar, the nonenzymatic reaction may proceed more rapidly due to the high yield in the formation of HAAs. However, at a higher level of sugar, the amino acids may not participate in the nonenzymatic browning reaction. On the contrary, the caramelization reaction may proceed rapidly because of the formation of a low yield

Table 11–Content of thermic HAAs in pork.

Sample	Method	Units	PhIP	IQ	MeIQ	IQx	8-MeIQx	7,8-DiMeIQx	4,8-DiMeiQx	Others	Ref.
–	HPLC-ESI-MS-MS-SRM	ng/g		<0.1	<0.1	<0.1		<0.1 to 0.3			(Richling and others 1998)
Heated, lyophilized, 180 to 220 °C/10 to 30 min	HPLC-API-ES-MS	ng/g	130 to 320	20 to 73			11 to 2		34 to 67	DMIP: 5 to 58	(Messner and Murkovic 2004)
275 °C/30 min	HPLC-FD-DAD	ng/g fresh wt	4.7 ± 4.1	<0.1	<0.1	<0.1	3.5 ± 1.1		0.4 ± 0.3	IFP: 2.5 ± 3.9 DMIP: 37 ± 46 TMIP: <0.1 4-MeIQX: <0.1	(Pais and others 1999)
275 °C/30 min, meat drippings			6.9 ± 3.3	<0.1	<0.1	<0.1	4.8 ± 0.4		2.2 ± 0.3	IFP: 13.4 ± 1.0 DMIP: 15.8 ± 4.4 TMIP: 4.0 ± 4.2 4-MeIQX: <0.1	
Fried, 200 °C	GC-NICI-MS-SIM	ng/g					nd to 0.4				(Tikkanen and others 1993)
Grilled			nd to 3.8				nd to 0.2				
Barbecued	GC-NICI-MS	ng/g	4.2				0.4		0.1		(Murray and others 1993)
Roasted (oven)	HPLC-UV	ng/g fresh wt	4.6	<0.1	<0.1		5.2	<0.1	1.5		(Zimmerli and others 2001)
Grilled/barbecued	HPLC-FD-DAD	ng/g	0.16				<0.1	nd	nd	IFP: nd	(Wong and others 2005)
Grilled/roasted			5.41				0.47	nd	0.33	IFP: 0.56	
Pan-fried			2.20				0.94	0.11	0.28	IFP: 0.31	
Stir-fried			0.15				nd	nd	nd	IFP: nd	
Deep-fried, or steamed/broiled, or stewed			nd				nd	nd	nd	IFP: nd	
longissimus dorsi muscle, grilled, 200 °C; nonaged; normal quality, internal temperature 70 °C, medium done	HPLC-API-ESI-MS-MS	ng/g	0.41 ± 0.44				0.61 ± 0.48		0.14 ± 0.12		(Polak and others 2009b)
"; internal temperature 95 °C, well done			2.05 ± 1.48				1.80 ± 0.83		0.66 ± 0.46		
longissimus dorsi muscle, grilled, 200 °C; nonaged; PSE quality, internal temperature 70 °C, medium done			0.52 ± 0.07				0.86 ± 0.17		0.15 ± 0.04		
"; internal temperature 95 °C, well done			1.63 ± 1.19				2.69 ± 1.54		0.59 ± 0.38		
longissimus dorsi muscle, grilled, 200 °C; 10 d aged; normal quality, internal temperature 70 °C, medium done			1.41 ± 0.62				1.34 ± 0.39		0.33 ± 0.11		
"; internal temperature 95 °C, well done			3.46 ± 1.57				3.87 ± 1.20		1.00 ± 0.24		
longissimus dorsi muscle, grilled, 200 °C; 10 d aged; PSE quality, internal temperature 70 °C, medium done			1.43 ± 0.31				1.98 ± 0.64		0.39 ± 0.12		
"; internal temperature 95 °C, well done			4.68 ± 2.02				5.82 ± 1.71		1.37 ± 0.46		
Blackened, 100 °C/10 to 17 min, Cajun style, well done	HPLC-FD-DAD	ng/g	3.0	<0.1			0.53		<0.1		(Knize and others 1996, 1997a, 1997b)
Belly, fried, 150 to 225 °C/4 to 8 min	HPLC-FD-DAD	ng/g	0.02 to 12.4	nd			nd to 2.9		nd to 0.7		(Skog and others 1995)
Belly, fried, 150 to 225 °C/4 to 8 min, pan residue			0.04 to 4.0	0.1			nd to 0.9		nd to 0.2		
Chop	HPLC-FD-DAD	ng/g	2.4				0.4		<0.1	IFP: <0.1 DMIP: <0.1 TMIP: <0.1	(Pais and others 2000)
Chop, fried, 150 to 225 °C/8 to 9.5 min	HPLC-FD-DAD	ng/g	nd to 4.8	nd			nd to 2.6		nd to 1.1		(Skog and others 1995)
Chop, fried, 150 to 225 °C/8 to 9.5 min, pan residue			0.02 to 3.8	0.1			nd to 1.9		nd to 0.5		
Chop, fried, 190 to 200 °C/15 min, well done	HPLC-DAD	ng/g	2.1	1.1	1.8		3.2		7.5		(Warzecha and others 2004)

(*Continued*)

Table 11–(*Continued*)

Sample	Method	Units	PhIP	IQ	MeIQ	IQx	8-MeIQx	7,8-DiMeIQx	4,8-DiMeiQx	Others	Ref.
Chop, fried, 176 °C/15 min, very well done	LC-ESI-MS-MS-SRM	ng/g	<0.03	<0.03		<0.03	<0.03		<0.03	IFP: <0.03 IQ[4,5-*b*]: 0.16 ± 0.02 IgQx: <0.03 7-MeIgQx: 0.37 ± 0.03 6,7-DiMeIgQx: <0.03 7,9-DiMeIgQx: <0.03	(Ni and others 2008)
Chop, fried, 200 °C/6 min	HPLC-DAD	ng/g	0.8 to 1.4				1.5 to 1.9		0.3 ± 0.1		(Olsson and others 2002)
Chop, fried, 200 °C/6 min, not carriers of RN⁻ allele			1.9 ± 0.3				1.9 ± 0.2		0.4 ± 0.1		
Chop, fried, 200 °C/6 min, carriers of RN⁻ allele			0.2 ± 0.3				1.5 ± 0.3		0.2 ± 0.1		
Chop, fried, 160 °C/6 min, not carriers of RN⁻ allele	LC-ESI-IT-MS-SIM	ng/g	0.08			nd	0.10				(Olsson and others 2005)
Chop, fried, 200 °C/6 min, not carriers of RN⁻ allele			3.27			nd	0.86				
Chop, fried, 160 °C/6 min, carriers of RN⁻ allele			0.05			nd	0.08				
Chop, fried, 200 °C/6 min, carriers of RN⁻ allele			0.14			0.19	0.16				
Chop, fried, 175 to 176 °C/5 to 15 min	HPLC-FD-DAD	ng/g	<0.2	<0.2	<0.2		<0.2 ng to 3.8		<0.2		(Sinha and others 1998a)
Chop, fried/cooked	HPLC-UV	ng/g fresh wt	<0.1 to 2.5	<0.1	<0.1		<0.1 to 2.3	<0.1	<0.1 to 0.6		9Zimmerli and others 2001)
Chop, fried, gravy			1.6 to 3.9	<0.1	<0.1		<0.1 to 1.1	<0.1	<0.1		
Chop, grilled			<0.1 to 0.9	<0.1	<0.1		<0.1 to 0.7	<0.1	<0.1		
Chop, fried, 170 °C/12 min, very well done	HPLC-DAD	ng/g	nd	nq	9.28 ± 0.82		4.58 ± 0.02		1.74 ± 0.39	DMIP: 2.40 ± 0.29	(Janoszka and others 2009)
Chop gravy, fried, 170 °C/12 min, very well done			1.52 ± 0.15	nq	10.54 ± 0.70		1.40 ± 0.11		1.62 ± 0.26	DMIP: nq	
Collar, fried, 170 °C/20 min, very well done			4.59 ± 0.72	nq	nq		2.62 ± 0.20		nq	DMIP: nq	
Collar gravy, fried, 170 °C/20 min, very well done			1.87 ± 0.18	nq	7.34 ± 0.63		1.02 ± 0.11		nq	DMIP: nd	
Fillet, fried, 150 to 225 °C/7 min	HPLC-UV/FD	ng/g	<0.4 ng to 13.4				<2 ng to 4.6		<4 ng to 3.3		(Skog and others 1997)
Fillet, fried, 150 to 225 °C/7 min, pan residue			0.3 to 32.0	0.1	0.1		0.06 to 5.6		0.08 to 4.2		
Fillet with bacon, fried/cooked	HPLC-UV	ng/g fresh wt	13.1	<0.1	<0.1		4.2	<0.1	1.1		(Zimmerli and others 2001)
Fillet, grilled, 15 + 15 min, very well done	HPLC-DAD	ng/g	11.7	6.7	<4 ng		9.5		28.2		(Warzecha and others 2004)
Joint, roasted, 240 °C/ 25 + 25 min, well done			1.3	<4 ng	<4 ng		nq		2.2		
Joint, ready to eat	HPLC-ESI-MS-MS-SRM	ng/g	0.4				1.6		0.3		(Richling and others 1998)
Knuckle, very well done	HPLC-FD	ng/g	0.1 to 1.5								(Ristic and others 2004)
Knuckle, very well done, crusts (fast food)	HPLC coulometric electrode array detect.	ng/g		3.7	7.4		5.9		2.1	DMIP: nq	(Gerbl and others 2004)
Loin, fatless, fried, 175 to 200 °C/10 min	LC-ESI-MS-MS-MRM	ng/g	2.5 ± 0.3	<0.1	<0.04		1.9 ± 0.9	0.4 ± 0.3	0.5 ± 0.2	DMIP: 3.9 ± 1.1	(Busquets and others 2004)
Loin, pan-fried, 180 to 200 °C/10 min	HPLC-ESI-IT-MS-MS	ng/g	2.5							4′-OH-PhIP: 0.7	(Busquets and others 2007)
Loin, coated-fried, 180 to 200 °C/7 min			1.3							<0.08	
Loin, coated-fried, 180 to 200 °C/7 min	HPLC-ESI-IT-MS-MS	ng/g	1.3 ± 0.3		nd		<0.3	nd	nd	DMIP: nd	(Busquets and others 2008)
Loin, griddled, 180 to 200 °C/10 min			2.1 ± 0.3		nd		0.5 ± 0.1	nd	0.1 ± 0.1	DMIP: 1.0 ± 0.3	
Loin, griddled	HPLC-MS	ng/g	258.2	nq				nq	nq	TriMeIQx: nd	(Back and others 2009)
Neck, roasted ("on salt"), 180 °C/3 h, well done	HPLC-DAD	ng/g	2.0	nq	nq		nq		nq		(Warzecha and others 2004)
Neck, grilled, 15 + 15 min, very well done			12.0	5.8	4.4		9.1		17.4		
Rib, boned, grilled, commercial	LC-ESI-MS	ng/g	5.2 to 17.2	nd	nd		10.6 to 20.8	nd	nd	TriMeIQx: nd	(Jo and others 2008)
Rib, grilled	HPLC-UV	ng/g fresh wt	<0.1	<0.1	<0.1		1.0	<0.1	<0.1		(Zimmerli and others 2001)

(*Continued*)

Table 11–(*Continued*)

Sample	Method	Units	PhIP	IQ	MeIQ	IQx	8-MeIQx	7,8-DiMeIQx	4,8-DiMeIQx	Others	Ref.
Rib, baked	HPLC-FD-DAD	ng/g	0.5 to 2.3	<0.1	<0.1		<0.1		<0.1		(Knize and others 1998)
Rib, smoked			0.7 to 7.4	<0.1	<0.1		<0.1		<0.1		
Rinds, 100 °C/10 to 17 min	HPLC-FD-DAD	ng/g	<0.1	<0.1			0.42		0.1		(Knize and others 1997b)
Raw pork, *longissimus dorsi* muscle	HPLC-UV-FD	ng/g	nd								(Liao and others 2009)
Pork floss, stir fried, 100 °C			nd								
Pork floss, stir fried, 125 °C			1.06 ± 0.22								
Pork floss, stir fried, 150 °C			4.97 ± 1.24								
Pork floss, stir fried, 125 °C, 0.1% vitamin C			0.73 ± 0.11								
Pork floss, stir fried, 125 °C, 0.1% vitamin E			0.28 ± 0.06								
Marinated, with 1% rock candy, without soy sauce	HPLC-DAD	ng/g	<0.03 ng	<0.13 ng	<0.15 ng		<0.30 ng		0.06 ± 0.02		(Lan and Chen 2002)
Marinated, with 1% rock candy and 5% to 20% soy sauce			1.22 to 1.34	0.71 to 0.82	0.24 to 0.44		1.24 to 1.44		1.93 to 2.04		
Marinated, with 10% soy sauce, without rock candy			1.23 ± 0.58	0.54 ± 0.35	0.07 ± 0.03		0.85 ± 0.26		1.02 ± 0.75		
Marinated, with 10% soy sauce and 0.5% to 5% rock candy			1.35 to 1.84	0.63 to 0.97	0.17 to 0.35		0.94 to 1.51		1.15 to 1.33		
Marinated, with 10% soy sauce and 1% rock candy, 1 to 4 h	HPLC-DAD	ng/g	1.33 to 2.53	0.74 to 1.28	0.44 to 0.64		1.44 to 3.43		1.85 to 2.32		(Lan and others 2004)
Marinated, with 10% soy sauce and 1% rock candy, 8 to 32 h			3.26 to 7.14	1.85 to 2.13	0.72 to 0.83		6.26 to 8.85		2.44 to 3.54		
Marinated, with 10% soy sauce and 1% rock candy, 1 h			1.26 ± 0.98	0.74 ± 1.28	0.30 ± 0.65		1.26 ± 1.43		1.28 ± 1.18		
Marinated, with 10% soy sauce and 1% rock candy, 1 h, with vitamin C			1.28 ± 1.50	0.72 ± 1.76	0.28 ± 1.14		1.31 ± 0.98		1.26 ± 0.59		
Marinated, with 10% soy sauce and 1% rock candy, 1 h, with α-tocopherol			1.36 ± 1.07	0.52 ± 0.83	0.28 ± 0.57		0.64 ± 1.16		0.64 ± 2.08		
Marinated, with 10% soy sauce and 1% rock candy, 1 h, with BHT			1.01 ± 2.46	0.72 ± 1.04	0.26 ± 0.75		1.23 ± 2.18		1.29 ± 3.17		
Pork juice, marinated, with 1% rock candy, without soy sauce	HPLC-DAD	ng/mL	0.04 ± 0.01	<0.13 ng	<0.153 ng		<0.30 ng		0.07 ± 0.03		(Lan and Chen 2002)
Pork juice, marinated, with 1% rock candy, with 5% to 20% soy sauce			1.47 to 2.66	0.93 to 1.73	0.74 to 0.93		1.85 to 3.03		1.46 to 3.75		
Pork juice, marinated with 10% soy sauce, without rock candy			1.33 ± 0.75	0.63 ± 0.58	0.17 ± 0.24		0.92 ± 1.10		1.14 ± 0.87		
Pork juice, marinated, with 10% soy sauce and 0.5% to 5% rock candy			2.32 to 2.64	0.96 to 1.52	0.42 to 0.70		1.63 to 2.02		1.41 to 1.83		
Pork juice, marinated, with 10% soy sauce and 0.5% to 5% rock candy, 1 to 4 h	HPLC-DAD	ng/g	2.47 to 3.66	1.52 to 1.74	0.77 to 0.84		1.82 to 3.47		1.48 to 3.53		(Lan and others 2004)
Pork juice, marinated, with 10% soy sauce and 0.5% to 5% rock candy, 8 to 32 h			5.35 to 7.36	1.88 to 2.23	0.83 to 0.93		5.44 to 9.15		3.62 to 3.92		
Boiled juice	HPLC	ng/g fresh wt		3.7	1.2		4.1				(Lee and others 1994)
Stewing cubes, fried, 150 to 225 °C/5 min	HPLC-UV-FD	ng/g	<0.4 ng to 0.1				<2 ng to 0.7		<4 ng to 0.2		(Skog and others 1997)
Stewing cubes, fried, 150 to 225 °C/5 min, pan residue			0.04 to 1.8				0.03 to 2.6		<0.01 to 0.6		
Sucking pig, ready to eat	HPLC-ESI-MS-MS-SRM	ng/g	1.0	<0.1	<0.1		0.8	0.2	0.4		(Richling and others 1998)

of HAAs. In addition, the Maillard reaction products may react with the mutagenic substances and result in a decrease of HAAs (Tai and others 2001). Similar results were obtained in a meat flavor model system in wet and dry conditions (Bordas and others 2004). In an open dry model system, the addition of glucose reduced the formation of IQx and 8-MeIQx and had no or only a slight enhancing effect on the formation of PhIP, harman, and norharman (Skog and others 2000).

In fresh pork sausage, the presence of a reducing sugar, dextrose, may decrease the formation of HAAs. This reduction may be due to a blocked reaction between creatinine and Maillard reaction intermediates, such as 5-hydroxymethyl-2-furfural (Abdulkarim and Smith 1998). In the same way, a possible relationship between brown sugar (sucrose) and 8-MeIQx formation in grilled chicken is supposed (Salmon and others 1997).

The effect of adding different oligosaccharides and inulin on the formation of HAAs and overall mutagenicity in fried ground patties were evaluated (Shin and others 2003a). A progressive reduction in the formation of HAAs was found when increasing the amounts of added oligosaccharides and inulin, in the range

Table 12–Content of pyrolytic HAAs in deer, lamb, rabbit, and reindeer meats.

Sample	Method	Units	Glu-P-2	Glu-P-1	Harman	Norharman	AαC	MeAαC	Trp-P-2	Trp-P-1	Ref.
Deer, oven-broiled, 200 °C/60 min	HPLC-FD	ng/g					0.04	0.03	0.2	0.1	(Ristic and others 2004)
Lamb chops, fried 225 °C/9 min	HPLC-UV/FD	ng/g			nq	nq			<0.3 ng	1.0	(Skog and others 1997)
Lamb chops, fried, 225 °C/9 min, pan residue					nq	nq			<0.3 ng	<0.3 ng	
Lamb steak, without fat and bones, griddled, 175 to 200 °C/11 min	LC-ESI-MS-MS-MRM	ng/g	<0.1	<0.1	7.2 ± 0.4	9.1 ± 0.5	0.5 ± 0.3	<0.2	<0.1	<0.1	(Busquets and others 2004)
Mutton, broiled, 6 min	HPLC-FD-DAD	ng/g			67.7	458					(Totsuka and others 1999)
Rabbit, boiled/60 min	HPLC-FD	ng/g					0.2	0.1	0.4	0.3	(Ristic and others 2004)
Reindeer fried, 225 °C/5 min	HPLC-UV/FD	ng/g			nq	nq			1.3	1.4	(Skog and others 1997)
Reindeer fried, 225 °C/5 min, pan residue					nq	nq			nd	nd	
Reindeer fried, crust, 225 °C/5 min	GC-NIC-MS-SIM	ng/g dry wt					<0.5 ng	<0.1 ng	nq	0.24	(Skog and others 1998b)

Table 13–Content of thermic HAAs in deer, horse, lamb, rabbit, and reindeer meats.

Sample	Method	Units	PhIP	IQ	MeIQ	IQx	8-MeIQx	7,8-DiMeIQx	4,8-DiMeIQx	Others	Ref.
Deer, oven-broiled, 200 °C/60 min	HPLC-FD	ng/g	0.2								(Ristic and others 2004)
Horse, steak, grilled	HPLC-UV	ng/g fresh wt	1.4	<0.1	<0.1		0.7	<0.1	<0.1		(Zimmerli and others 2001)
Lamb, fried/cooked			<0.1 to 1.0	<0.1	<0.1		<0.1 to 0.4	<0.1	<0.1 to 1.0		
Lamb, grilled			<0.1 to 9.7	<0.1	<0.1		1.5 to 2.8	<0.1	0.7		
Lamb, grilled, well done	HPLC-FD-DAD	ng/g	11				1.6		<0.1		(Knize and others 1997a)
Lamb chops, fried, 150 to 225 °C/9 min	HPLC-UV/FD	ng/g	nd to 1.5				<2 ng to 0.4		<4 ng to 0.6		(Skog and others 1997)
Lamb chops, fried, 150 to 225 °C/9 min, pan residue			<0.01 to 2.3				0.08 to 0.6		0.04 to 0.3		
Lamb steak, griddled, without fat and bones, 175 to 200 °C/11 min	LC-ESI-MS-MS-MRM	ng/g	5.8 ± 0.2	<0.04	<0.04		1.3 ± 0.3	<0.1	1.8 ± 0.9	DMIP: <0.01	(Busquets and others 2004)
Lamb, sausage (Merguez)	HPLC-UV or HPLC-ESI-MS	ng/g	nd			0.7	1.8	0.1	1.9		(Fay and others 1997)
Rabbit, boiled/60 min	HPLC-FD	ng/g	1.4								(Ristic and others 2004)
Reindeer fried, 150 to 225 °C/5 min	HPLC-UV/FD	ng/g	0.4 to 5.8				<2 ng to 1.0		<4 ng		(Skog and others 1997)
Reindeer fried, 150 to 225 °C/5 min, pan residue			nd to 3.5				0.1 to 0.8		0.03 to 0.6		

0.5 to 2.5 g/100 g sample. The addition of amounts higher than 1.5% inhibited total formation of HAAs by more than 46%, but no significant difference was detected between the percentages of inhibition achieved with 1.5% to 2.5% of these compounds. Besides, the inhibition of the formation of HAAs is accompanied by a concomitant reduction in the overall mutagenicity of the patties (Shin and others 2003a). Similar results were found when buckwheat, clover, and sage honeys were added to ground beef patties (Shin and others 2003b). The carbohydrates present in honey are the primary inhibitory compounds in the formation of HAAs, as it has been demonstrated by addition of fructose, glucose, or fructose and glucose together to ground beef before cooking (Shin and others 2003b). In the same way, mutagenic activity is inhibited (34% to 76%) by adding glucose, lactose, or powdered milk to beef patties before frying. However, starch from golden bread crumbs only caused a nonsignificant decrease in mutagenic activity, whereas adding both starch and glucose to the beef patties inhibited mutagenic activity by up to 54% (Skog and others 1992a). Starch from potatoes inhibited the formation of HAAs (Skog and others 1992a; Persson and others 2004) and potato fiber affected mainly the formation of PhIP (Persson and others 2004).

Effect of amino acids. It has been reported that proline (Pro) or tryptophan (Try) can compete in the reaction of creatinine with precursors of HAAs. Phe is a precursor of PhIP, and Try has been confirmed as an important precursor of the β-carbolines, which are very easily formed at normal cooking temperatures (Skog and others 2000). However, Try does not enhance the formation of α- or γ-carbolines. It enhances the formation of IQx compounds and inhibits PhIP formation (Skog and others 2000).

In the presence of different levels of sugar, the contents of free amino acids in fried fish fiber were found to decrease with increasing yields of HAAs (Tai and others 2001). For various amino acids, a high correlation was found between the consumption of Phe, leucine (Leu), serine (Ser), or threonine (Thr) and the amounts of HAAs formed. Likewise, a high correlation was found between the consumption of glucose and the amounts of HAAs formed. It may be postulated that amino acids may play a more significant role in the formation of HAAs in fried fish fiber than glucose (Tai and others 2001). A high amount of glycine (Gly), alanine, and Phe, 50 times the native one, favored the formation of 8-MeIQx and PhIP in wet and in dry conditions. The formation of other HAAs was diminished (Bordas and others 2004). The concentration of PhIP formed in the models corresponded with the content of Phe, Leu, isoleucine (Ileu), and tyrosine (Tyr), except for the beef and the pork (Pais and others 1999). The unexpectedly low PhIP concentrations in beef and pork may be explained by the sugar content

Table 14–Content of pyrolytic HAAs in chicken.

Sample	Method	Units	Glu-P-2	Glu-P-1	Harman	Norharman	AαC	MeAαC	Trp-P-2	Trp-P-1	Ref.
Heated, lyophilized, 180 to 220 °C/10 to 30 min	HPLC-API-ES-MS	ng/g					nq to 58	nq to 1.7	nq	nq to 220	(Messner and Murkovic 2004)
Meat, boiling, 100 °C/240 min	HPLC-UV/FD	ng/g			0.2	0.4	nd	nd	nd	nd	(Solyakov and Skog 2002)
Meat, barbecued, commercial					1.5 to 1.8	1.5	nd	nd	nd	nd	
Without skin and boneless, barbecued; 260 °C/40 min, well done	LC-ESI-MS-MS-SRM	ng/g					5.38 ± 0.03	0.66 ± 0.06			(Ni and others 2008)
";260 °C/43 min, very well done							40.36 ± 6.98	6.12 ± 0.76			
Without skin and boneless, oven-broiled; 86 °C/9 min, just done							0.18 ± 0.03	<0.03			
"; 79 °C/14 min, well done							2.54 ± 0.17	0.36 ± 0.02			
"; 83 °C/17 min, very well done							9.43 ± 1.27	1.31 ± 0.25			
Without skin and boneless, fried; 197 °C/14 min, just done							<0.03	<0.03			
"; 202 °C/28 min, well done							<0.03	<0.03			
"; 211 °C/36 min, very well done							0.07 ± 0.01	<0.03			
Commercial	HPLC-ESI-MS-MS-SRM	ng/g	<0.1	nq							(Richling and others 1998)
Broiled, 6 min	HPLC-FD-DAD	ng/g			133	622					(Totsuka and others 1999)
Very well done	HPLC-FD	ng/g					0.1	0.05	0.2	0.04	(Ristic and others 2004)
Chicken Fajita, 100 °C/10 to 17 min	HPLC-FD-DAD	ng/g					<0.1				(Knize and others 1996, 1997b)
Breast, boiled, 100 °C/23 min	HPLC-UV/FD	ng/g			0.1	nd	nd	nd	nd	nd	(Solyakov and Skog 2002)
Breast, pan-fried, 140 to 220 °C/12 to 34 min					0.1 to 6.9	0.3 to 7.5	nd	nd	nd	nd	
Breast, pan-residue, 170 to 220 °C/12 to 18 min					nq	nq to 0.1	nd	nd	nd	nd	
Breast, fried, 175 °C/30 min	LC-ESI-IT-MS-SIM	ng/g			0.1	0.2	0.08	nd	0.3	nd	(Bang and others 2002)
Breast, pan-fried, 190 °C/12 min	HPLC-FD-DAD	ng/g			nd	nd	nd	6.8	5.1	3.7	(Brockstedt and Pfau 1998)
Breast, fried	LC-APCI-MS-MS	ng/g					nd				(Holder and others 1997)
Breast, fried, 150 to 225 °C/30 min	HPLC-UV/FD	ng/g			nq	nq	nq	nq	<0.3 ng	<0.3 ng to 1.6	(Skog and others 1997)
Breast, fried, 150 to 225 °C/30 min, pan-residue					nq	nq	nq	nq	<0.3 ng	<0.3 ng to 0.02	
Breast without fat, pan-fried, 220 °C/5 min	LC-ESI-MS-MS	ng/g			6.4 ± 0.2	19 ± 2					(Busquets and others 2006)
Breast without skin, fat and bones, fried,175 to 200 °C/12 min	LC-ESI-MS-MS-MRM	ng/g	<0.1	<0.1	7.5 ± 0.3	15.1 ± 0.2	<1.0	<0.02	<0.02	<0.2	Busquets and others 2004
Breast without skin, fat and bones, griddled, 175 to 200 °C/13 min			<0.1	<0.1	1.1 ± 0.1	3.1 ± 0.04	0.2 ± 0.2	<0.02	<0.02	<0.02	
Breast, fried, 170 °C/3 min	LC-ESI-IT-MS	ng/g	nq	nq	0.5 ± 0.03	0.3 ± 0.06	nq	nq	nq	nq	(Bermudo and others 2005)
	LC-ECD		nq	nq	0.5 ± 0.03	0.3 ± 0.05	nq	nq	nq	nq	
Breast, fried, 180 °C/5 min	LC-ESI-IT-MS		nq	nq	1.5 ± 0.4	1.5 ± 0.06	nq	nq	nq	nq	
	LC-ECD		nq	nq	1.8 ± 0.1	1.5 ± 0.2	nq	nq	nq	nq	
Breast, griddled, 180 °C/5 min	LC-ESI-IT-MS		nq	nq	0.4 ± 0.01	nq	nq	nq	nq	nq	
	LC-ECD		nq	nq	0.4 ± 0.01	nq	nq	nq	nq	nq	
Breast, roasted, 140 to 160 °C/138 min	HPLC-ESI-IT-MS-MS	ng/g			0.9 ± 0.4	0.9 ± 0.3	nd	nd	<0.3	<0.2	(Busquets and others 2008)
Breast, coated-fried, 180 to 200 °C/9 min					1.3 ± 0.4	9.7 ± 0.5	nd	nd	nd	<0.1	
Breast, grilled/6 min	LC-APCI-MS-MS	ng/g					<104				(Holder and others 1997)
Breast, fried in soybean oil, commercial	LC-ESI-MS	ng/g	nd	nd	nd to 32.8	nd to 38.1	nd to 14.2	3.61 to 27.7	nd to 4.72	nd	(Jo and others 2008)

(*Continued*)

Table 14–(*Continued*)

Sample	Method	Units	Glu-P-2	Glu-P-1	Harman	Norharman	AαC	MeAαC	Trp-P-2	Trp-P-1	Ref.
Breast, Charcoal-grilled, commercial			nd	nd	nd to 50.0	nd to 103	nd	nd to 15.6	nd	nd	
Breast, electrical grilled, commercial			nd	nd	nd to 92.3	nd to 261	17.3 to 58.0	nd to 12.2	nd	nd	
Breast without bones and skin, barbecued 230 to 300 °C/10 min	HPLC-ESI-MS-MS-SRM	ng/g					8.70 ± 1.29	0.23 ± 0.08			(Turesky and others 2007)
Breast with bone and skin, barbecued 191 °C/63 min, well done	LC-ESI-MS-MS-SRM	ng/g					4.09 ± 0.43	0.47 ± 0.07			(Ni and others 2008)
Breast, 275 °C/30 min	HPLC-FD-DAD	ng/g fresh wt	<0.1	<0.1	5.2	0.27	<0.1	<0.1	<0.1		(Pais and others 1999)
Breast, meat drippings, 275 °C/30 min			<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.05 ± 0.03	
Breast, roasted, 150 °C/30 min, pan residue	HPLC-FD-DAD	ng/g			nq	nq			nd	0.02	(Skog and others 1995)
Breast, roasted, 175 to 245 °C/25 to 40 min	HPLC-UV/FD	ng/g			nq to 3.3	nd to 1.7	nd	nd	nd	nd	(Solyakov and Skog 2002)
Legs, fried, 200 °C/10 min	HPLC-FD-DAD	ng/g	nd	nd	0.12	0.10	0.13	nd	0.14	0.18	(Chen and Yang 1998)
Legs, deep-fried, 100 to 200 °C/5 to 15 min	HPLC-FD-DAD	ng/g fresh wt			nd to 2.11	nd to 1.31	nd to 0.23	nd	nd to 0.49	0.09 to 0.97	(Chiu and others 1998)
Legs with skin, microwaved, 2450 MHz/5 to 15 min					nd to 1.73	nd to 1.11	nd to 0.15	nd to 0.14	nd to 0.14	0.10 to 0.16	
Legs skinless, microwaved, 2450 MHz/5 to 15 min					nd to 2.84	nd to 0.91	0.10 to 0.14	nd	nd to 0.18	0.11 to 1.03	
Liver, pan-fried, 190 °C/9 min	HPLC-UV/FD	ng/g			nd	nd	nd	nd	nd	nd	(Solyakov and Skog 2002)
Skin, barbecued, commercial					6.5 to 9.6	10.3 to 12.9	nd	nd	nd	nd	
Thigh, 275 °C/30 min	HPLC-FD-DAD	ng/g fresh wt			8.3	12.9					(Pais and others 1999)
Wok, fried/3 min	HPLC-FD	ng/g					<0.02	<0.02	0.1	0.1	(Ristic and others 2004)

in these meats. Furthermore, the level of creatine and some free amino acids in the pork meat were affected by the RN genotype of the pigs (Olsson and others 2002). The meat of the carrier of the RN^- allele had lower levels of intramuscular fat, free amino acids, and muscular ultimate pH, and considerably higher dripping loss, creatine, dipeptides, and residual glycogen content than that of the noncarrier (rn^+/rn^+) (Olsson and others 2005). In these samples, and for all frying temperatures, more PhIP was formed in the rn^+/rn^+ meat with lower levels of residual glycogen, than in the RN^-/rn^+ meat that has higher levels of glycogen. IQx was only formed in chops of the carrier of the RN^- allele and only when the initial frying temperature was higher than 160 °C. Harman and norharman were only formed in chops from the noncarrier. The concentration of reducing sugars is clearly related to the surface browning of pig meat, and the higher residual glycogen content in the RN^-/rn^+ meat probably explains the darker crust colors through a more pronounced Maillard reaction (Olsson and others 2005).

The contents of 5 HAAs have been studied according to their dependence on aging time (as nonaged, 24 h *post mortem compared with* aged, 3, 6, and 10 d *post mortem*), muscle quality (normal *compared with* PSE [pale, soft, and exudative]), and internal cooking temperature of grilled pork (Polak and others 2009b). In the comparison with red firm nonexudative (normal) quality, pork meat may show extreme such as PSE quality, originated in rapid *post mortem* glycolysis, slow carcass chilling rates, or a combination of both. In PSE quality muscle, there was a lower level of free amino acids but higher a level of glucose. Creatine content decreased significantly with days of aging, but creatinine, free amino acids, and glucose increased (Polak and others 2009a, 2009b). The content of HAAs increased with aging stage (1 to 10 or 14 d) and was dependent on internal temperature (Polak and others 2009a, 2009b). The higher content of free amino acids, as a consequence of muscle quality and aging time, was associated with the higher content of HAAs of the grilled pork, with a quite strong correlation between these parameters (Polak and others 2009b).

On the other hand, pork floss is a traditional meat product consumed in China. It is often prepared by steaming raw pork for 3 to 4 h, followed by pressing, tearing, and then adding several additives, such as sugar, salt, monosodium glutamate, edible oil, soybean flour, and antioxidants. After subjecting the cooked pork to stir-frying for about 1 to 2 h, a brown-colored pork product in a shredded form is obtained. For steaming pretreatment, the content of both glucose and free amino acids in pork floss showed a decreased trend for increasing temperature (Liao and others 2009). On the contrary, the level of HAAs formed showed an increasing order. This result suggests that glucose and free amino acids contribute to formation of HAAs during processing. The reduction of glucose and free amino acids was correlated with the formation of HAAs, and correlation coefficients were $r = 0.93$ and $r = 0.97$, respectively. Sugar can enhance or inhibit the formation of HAAs in pork floss depending on the proportion of the reactants. The total amount of free amino acids decreased with increasing temperature. This result indicates that some free amino acids (glutamic acid, Ileu, Tyr, Thr, Ser, and Phe) degrade by themselves or react with glucose, which leads to the formation of HAAs in pork floss. The content or Try was not determined (Liao and others 2009).

Effect of monosodium glutamate (MSG). The effect of MSG on the formation of apolar HAAs was studied in fried fish fiber (Tai

Table 15–Content of thermic HAAs in chicken.

Sample	Method	Units	PhIP	IQ	MeIQ	IQx	8-MeIQx	7,8-DiMeIQx	4,8-DiMeIQx	Others	Ref.
Roasted, read to eat	HPLC-ESI-MS-MS-SRM	ng/g	2.4 to 5.3	<0.1	<0.1		2.2 to 3.2	<0.1	1.3 to 2.0	TriMeIQx: <0.1	(Richling and others 1998)
Nuggets, deep-fried, read to eat			0.1	<0.1	<0.1		0.2	<0.1	0.4	TriMeIQx: <0.1	
Commercial (fast food)	HPLC-FD-DAD	ng/g	<0.1				<0.1		<0.1		(Knize and others 1995)
Very well done	HPLC-FD	ng/g	11.1								(Ristic and others 2004)
Heated, lyophilized, 180 to 220 °C/10 to 30 min	HPLC-API-ES-MS	ng/g	76 to 320	nq to 48			nq to 17		nq to 39	DMIP: 8.5 to 15	(Messner and Murkovic 2004)
Steamed/boiled	HPLC-FD-DAD	ng/g	nd				nd	nd	nd	IFP: nd	(Wong and others 2005)
Meat, boiling, 100 °C/240 min	HPLC-UV/FD	ng/g	nd	nd	nd	nd	nd	nd	nd		(Solyakov and Skog 2002)
Meat, barbecued, commercial			nd	nd	nd	nd	nq	nd	nd		
Skin, barbecued, commercial			nd to 0.8	nd	nd	nd	1.1 to 2.3	nd	0.2 to 1.0		
Without skin and boneless, barbecued; 260 °C/40 min, well done	LC-ESI-MS-MS-SRM	ng/g	78.52 ± 4.83	0.64 ± 0.09		<0.03	1.98 ± 0.05		1.42 ± 0.02	IFP: 5.97 ± 0.77 IQ[4,5-*b*]: <0.03 IgQx: 0.80 ± 0.13 7-MeIgQx: 7.71 ± 0.28 6,7-DiMeIgQx: <0.037 7,9-DiMeIgQx: 1.12 ± 0.10	(Ni and others 2008)
";260 °C/43 min, very well done			304.71 ± 10.89	0.91 ± 0.11		<0.03	7.70 ± 1.15		5.52 ± 0.48	IFP: 25.56 ± 1.66 IQ[4,5-*b*]: <0.03 IgQx: <0.03 7-MeIgQx: 11.28 ± 2.38 6,7-DiMeIgQx: <0.03 7,9-DiMeIgQx: 2.89 ± 0.79	
Without skin and boneless, oven-broiled; 86 °C/9 min, just done			5.60 ± 0.09	0.07 ± 0.04		<0.03	0.13 ± 0.01		0.11 ± 0.01	IFP: 0.39 ± 0.02 IQ[4,5-*b*]: <0.03 IgQx: 0.20 ± 0.09 7-MeIgQx: 1.02 ± 0.06 6,7-DiMeIgQx: <0.03 7,9-DiMeIgQx: 0.08 ± 0.01	
"; 79 °C/14 min, well done			31.83 ± 3.65	0.15 ± 0.09		<0.03	0.82 ± 0.04		0.52 ± 0.02	IFP: 2.61 ± 0.18 IQ[4,5-*b*]: 0.34 ± 0.01 IgQx: 0.44 ± 0.07 7-MeIgQx: 3.64 ± 0.24 6,7-DiMeIgQx: 0.24 ± 0.01 7,9-DiMeIgQx: 0.55 ± 0.07	
"; 83 °C/17 min, very well done			71.96 ± 4.17	0.30 ± 0.00		<0.03	2.81 ± 0.20		1.98 ± 0.33	IFP: 11.33 ± 0.62 IQ[4,5-*b*]: <0.03 IgQx: 0.29 ± 0.03 7-MeIgQx: 11.10 ± 0.32 6,7-DiMeIgQx: <0.03 7,9-DiMeIgQx: 1.81 ± 0.13	
Without skin and boneless, fried; 197 °C/14 min, just done			8.77 ± 0.03	0.18 ± 0.17		0.07 ± 0.00	0.58 ± 0.02		0.78 ± 0.03	IFP: 2.66 ± 0.10 IQ[4,5-*b*]: <0.03 IgQx: 0.25 ± 0.03 7-MeIgQx: 1.48 ± 0.14 6,7-DiMeIgQx: 0.06 ± 0.01 7,9-DiMeIgQx: 0.24 ± 0.06	

(*Continued*)

Table 15–(*Continued*)

Sample	Method	Units	PhIP	IQ	MeIQ	IQx	8-MeIQx	7,8-DiMeIQx	4,8-DiMeIQx	Others	Ref.
"; 202 °C/28 min, well done			19.47 ± 0.39	0.11 ± 0.01		0.10 ± 0.01	1.25 ± 0.07		1.57 ± 0.23	IFP: 5.63 ± 0.25 IQ[4,5-*b*]: 0.24 ± 0.01 IgQx: 0.62 ± 0.05 7-MeIgQx: 5.07 ± 0.13 6,7-DiMeIgQx: 0.22 ± 0.01 7,9-DiMeIgQx: 0.63 ± 0.04	
"; 211 °C/36 min, very well done			48.54 ± 2.83	0.21 ± 0.01		0.21 ± 0.02	2.34 ± 0.08		3.61 ± 0.19	IFP: 15.88 ± 0.64 IQ[4,5-*b*]: <0.03 IgQx: 0.89 ± 0.19 7-MeIgQx: 8.65 ± 0.42 6,7-DiMeIgQx: <0.03 7,9-DiMeIgQx: 0.90 ± 0.10	
Roasted and barbecued	HPLC-FD-DAD	ng/g	0.12				<0.1	nd	nd	IFP: nd	(Wong and others 2005)
Barbecued	GC-NICI-MS	ng/g					0.3		0.1		(Murray and others 1993)
Casserole (commercial)			nd				nd		nd		
Grilled (fast food)	HPLC-FD-DAD	ng/g	<0.1 to 1.44	<0.1	<0.1		0.27 to 0.72		<0.1		(Knize and others 1998)
Grilled, chain restaurants	HPLC-MS-MS	ng/g	0.08 to 43,2								(Sullivan and others 2008)
Grilled	GC-NICI-MS-SIM	ng/g	nd to 1.1				nd to 0.12		nd to 0.08		(Tikkanen and others 1993)
Grilled, 220 °C/40 min	GC-NICI-MS-SIM	ng/g	1.4 to 7.6				0.11		0.10 to 0.15		(Tikkanen and others 1996)
Grilled, 220 °C/40 min, marinated			0.3 to 3.4				0.10 to 0.94		0.06 to 0.67		
Roast, 175 °C	HPLC-FD-DAD	ng/g	nd	nd	nd		nd		nd		(Sinha and others 1995)
Stew, 100 °C			nd	nd	nd		nd		nd		
Deep-fried	HPLC-FD-DAD	ng/g	1.75				0.36	0.15	0.23	IFP: nd	(Wong and others 2005)
Pan-fried			0.41				1.26	nd	0.48	"	
Stir-fried or stewed			nd				<0.1	nd	nd	"	
Pan-fried, skinless, well done	HPLC-UV/FD	ng/g	0.20 to 17.54				0.11 to 2.27		<2.26	IFP: 6.5	(Norrish and others 1999)
Chicken Fajita, 100 °C/10 to 17 min, well done, Mexican style	HPLC-FD-DAD	ng/g	6.4	<0.1			0.54		<0.1		(Knize and others 1996; 1997a, 1997b)
Breast, boiled, 100 °C/23 min	HPLC-UV/FD	ng/g	nd	nd	nd	nd	nd	nd	nd		(Solyakov and Skog 2002)
Breast, deep-fried, 160 °C/11 min			nq	nd	nd	nd	nq	nd	nq		
Breast, pan-fried, 140 to 220 °C/12 to 34 min			nd to 38.2	nd	nd	nd	0.1 to 1.8	nd	0.1 to 0.6		
Breast, 170 to 220 °C/12 to 18 min, pan-residue			nq to 0.2	nd	nd	nd	nd	nd	nd		
Breast, fried, 175 °C/30 min	LC-ESI-IT-MS-SIM	ng/g	0.4	nd	nd	nd	0.1	nd	0.07	IFP: 0.04 DMIP: nd	(Bang and others 2002)
Breast, pan-fried, 190 °C/12 min	HPLC-FD-DAD	ng/g	nd				nd	0.9	1.9		(Brockstedt and Pfau 1998)
Breast, fried, 180 to 200 °C/11 min	HPLC-IT-ESI-MS-MS	ng/g	19.2 ± 12.3							4′-OH-PhIP: 43.7 ± 13.8	(Busquets and others 2007)
Breast, coated-fried, 180 to 200 °C/9 min			0.6							4′-OH-PhIP: 0.3	
Breast, roasted, 140 to 160 °C/138 min	HPLC-IT-ESI-MS-MS	ng/g	0.9 ± 0.3		<0.1		<0.1	<0.05	<0.04	DMIP: <0.04	(Busquets and others 2008)
Breast, coated-fried, 180 to 200 °C/9 min			0.6 ± 0.4		<0.1		<0.2	nd	0.1 ± 0.4	DMIP: nd	
Breast, pan-fried	LC-APCI-MS-MS	ng/g	0.1	nd			nd				(Holder and others 1997)

(*Continued*)

Table 15–(*Continued*)

Sample	Method	Units	PhIP	IQ	MeIQ	IQx	8-MeIQx	7,8-DiMeIQx	4,8-DiMeIQx	Others	Ref.
Breast, fried, 160 to 220 °C/15 to 25 min	HPLC-FD	ng/g	0.5 to 19.4	<1	<1		<1 to 10.4				(Krul and others 2000)
Breast, pan-fried, 175 to 225 °C	HPLC	ng/g	0.04 to 3.0								(Persson and others 2002)
Breast, deep-fried, 175 to 225 °C			0.02								
Breast, fried, 175 to 225 °C/30 min	HPLC-UV/FD	ng/g	0.5 to 10.0				0.4 to 0.5		0.2 to 0.5		(Skog and others 1997)
Breast, fried, 150 to 225 °C/30 min, pan-residue			0.02 to 1.0		0.3		0.08 to 0.6		<4 ng to 0.3		
Breast without skin and bone, pan-fried, 197 to 211 °C/14 to 36 min	HPLC-FD-DAD	ng/g	12 to 70	<0.2	<0.2		1 to 3		1 to 4		(Sinha and others 1995)
Breast with skin and bone, pan-fried,190 °C/62 min			25	<0.2	<0.2		<0.2		<0.2		
Breast without fat, pan-fried, 220 °C/5 min	LC-ESI-MS-MS	ng/g	72 ± 9				2.3 ± 0.3		2.3 ± 0.03		(Busquets and others 2006)
Breast without skin, fat and bone, fried, 175 to 200 °C/12 min	LC-ESI-MS-MS-MRM	ng/g	46.9 ± 2.1	<0.04	<0.04		<0.1	<0.1	0.8 ± 0.3	DMIP: 29.7 ± 2.8	(Busquets and others 2004)
Breast without skin, fat and bone, griddled, 175 to 200 °C/13 min			2.3 ± 0.5	<0.04	<0.1		0.3 ± 0.1	<0.04	0.4 ± 0.1	DMIP: 1.9 ± 0.1	
Breast, fried, 170 °C/3 min	LC-ESI-IT-MS	ng/g	2.8 ± 0.05	nq	nq		0.3 ± 0.02	nq	0.12 ± 0.02	DMIP: 8.0 ± 0.4	(Bermudo and others 2005)
	LC-ECD		2.7 ± 0.1	nq	nq		nq	nq	nq	DMIP: 7.5 ± 0.4	
Breast, fried, 180 °C/5 min	LC-ESI-IT-MS		6.1 ± 0.2	0.6 ± 0.03	0.2 ± 0.02		1.2 ± 0.1	nq	0.5 ± 0.03	DMIP: 10.6 ± 1.9	
	LC-ECD		6.3 ± 0.7	0.6 ± 0.05	nq		1.1 ± 0.04	nq	0.5 ± 0.04	DMIP: 8.6 ± 0.6	
Breast, griddled, 180 °C/5 min	LC-ESI-IT-MS		3.4 ± 0.04	nq	nq		0.3 ± 0.01	nq	0.3 ± 0.01	DMIP: 5.2 ± 0.8	
	LC-ECD		3.5 ± 0.1	nq	nq		0.3 ± 0.02	nq	0.3 ± 0.04	DMIP: 4.5 ± 0.2	
Breast, griddled, 180 to 200 °C/13 min	LC-IT-MS-MS	ng/g	5.8 ± 2.7							4′-OH-PhIP: 13.4 ± 3.1	(Busquets and others 2007)
Breast, fried/cooked	HPLC-UV	ng/g fresh wt	<0.1 to 2.5	<0.1	<0.1		<0.1	<0.1	<0.1		(Zimmerli and others 2001)
Breast without skin and bone, fried, 200 °C	HPLC-UV/FD	ng/g	1.7 ± 0.6								(Moonen and others 2004)
Breast without skin and bone, fried, 200 °C, and oven-broiled, 250 °C/16 min			7.4 ± 2.0								
Breast, grilled/6 min	LC-APCI-MS-MS	ng/g	<226	<5			100				(Holder and others 1997)
Breast, grilled, 15 + 15 min, very well done	HPLC-DAD	ng/g	7.4	<0.4	4.9		1.8		2.1		(Warzecha and others 2004)
Breast, grilled, (up to 350 °C), well done	HPLC-FD-DAD	ng/g	21 to 270	<0.1			<0.1 to 0.63		0.53 to 3.1		(Knize and others 1996, 1997a)
Breast, fried in soybean oil, commercial	LC-ESI-MS	ng/g		nd			6.45 to 9.69	nd			(Jo and others 2008)
Breast, Charcoal-grilled, commercial				nd			6.87 to 14.7	nd			
Breast, electrical grilled, commercial				nd			nd	nd to 17.3			
Breast without bones and skin, barbecued, 230 to 300 °C/10 min	HPLC-ESI-MS-MS-SRM	ng/g	10.00 ± 0.06	0.118 ± 0.01		<0.03	0.34 ± 0.07		0.280 ± 0.05	7,9-DiMeIgIQx: 0.090 ± 0.026	(Turesky and others 2007)
Breast without skin and bone, grilled/barbecued, 177 to 260 °C/10 to 43 min	HPLC-FD-DAD	ng/g	27 to 480	<0.2	<0.2		<0.2 to 9		<0.2 to 2		(Sinha and others 1995)
Breast with skin and bone, grilled/barbecued, 191 °C/63 min			36	<0.2	<0.2		<0.2		<0.2		
Breast without skin and bone, oven-broiled, 79 to 86 °C/9 to 17 min			6 to 150	<0.2	<0.2		<0.2 to 3		<0.2		

(*Continued*)

Table 15–(*Continued*)

Sample	Method	Units	PhIP	IQ	MeIQ	IQx	8-MeIQx	7,8-DiMeIQx	4,8-DiMeIQx	Others	Ref.
Breast with skin and bone, oven-broiled, 180 °C/43 min			131	<0.2	<0.2		<0.2		<0.2		
Breast with bone and skin, barbecued 191 °C/63 min, well done	LC-ESI-MS-MS-SRM	ng/g	19.07 ± 1.09	0.13 ± 0.02		<0.03	0.63 ± 0.14		0.32 ± 0.06	IFP: 1.09 ± 0.01 IQ[4,5-*b*]: 0.36 ± 0.06 IgQx: 0.24 ± 0.02 7-MeIgQx: 2.76 ± 0.68 6,7-DiMeIgQx: 0.18 ± 0.01 7,9-DiMeIgQx: 0.55 ± 0.11	(Ni and others 2008)
Breast, broiled	HPLC	ng/g	0.07								(Persson and others 2002)
Breast, roasted, 140 to 160 °C/138 min	HPLC-ESI-IT-MS-MS	ng/g	0.9							4′-OH-PhIP: <0.08	(Busquets and others 2007)
Breast, roasted, 150 to 200 °C/30 min	HPLC-UV/FD	ng/g	0.04 to 0.3				nq	nq			(Skog and others 1997)
Breast, roasted, 150 to 200 °C/30 min, pan residue			0.09 to 0.6				nd to 0.02	nd to 0.01			
Breast, 275 °C/30 min	HPLC-FD-DAD	ng/g fresh wt	37.5 ± 15.4	<0.1	<0.1	<0.1	0.5 ± 0.1		0.2 ± 0.08	IFP: 7.0 ± 6.6 DMIP: 5.9 ± 5.2 4-MeIQx: <0.1 TMIP: 2.9 ± 3.5	(Felton and others 1999, Pais and others 1999)
Breast, 275 °C/30 min, meat drippings			3.3 ± 1.4	<0.1	<0.1	<0.1	0.1 ± 0.1	<0.1	0.3 ± 0.2	IFP: 1.8 ± 1.0 DMIP: 2.1 ± 0.6 4-MeIQx: <0.1 TMIP: <0.1	
Breast, roasted, 175 to 245 °C/25 to 40 min	HPLC-UV/FD	ng/g	nd to 3.0	nd	nd	nd	nd to 1.7	nd	nd to 0.3		(Solyakov and Skog 2002)
Breast, marinated with wine 0.5 to 3h; 35 mM Trolox∗	LC-IT-MS-MS	percent reduct	63 to 81							4′-OH-PhIP: 51 to 52	(Busquets and others 2007)
42 mM Trolox			36 to 47							22 to 32	
47 mM Trolox			22 to 74							2to 28	
Breast, marinated with wine 24 h; 35 mM Trolox			83							69	
42 mM Trolox			88							45	
47 mM Trolox			87							50	
Legs, fried, 200 °C/10 min	HPLC-FD-DAD	ng/g	0.21	0.10	0.17	0.11	0.13	0.16	0.09		(Chen and Yang 1998)
Legs, deep-fried, 100 to 200 °C/5 to 15 min	HPLC-FD-DAD	ng/g fresh wt	nd to 2.81	0.09 to 0.51	nd to 0.51	nd to 1.21	0.08 to 0.91	nd to 0.87	nd to 0.78		(Sinha and others 1995)
Legs with skin, microwaved, 2450 MHz/5 to 15 min			nd	nd	nd	nd	nd	nd	nd		
Legs skinless, microwaved, 2450 MHz/5 to 15 min			nd	nd	nd	nd	nd	nd	nd		
Liver, pan-fried, 190 °C/9 min	HPLC-UV/FD	ng/g	nd	nd	nd	nd	nd	nd	nd		(Solyakov and Skog 2002)
Thigh, 275 °C/30 min	HPLC-FD-DAD	ng/g fresh wt	8.0 ± 4.7	<0.1	<0.1	<0.1	0.02 ± 0.004		0.05 ± 0.03	IFP: 1.3 ± 1.6 DMIP: 3.1 ± 3.2 4-MeIQx: <0.1 TMIP: <0.1	(Pais and others 1999)
Thigh, 275 °C/30 min, meat drippings			21.8 ± 5.1	<0.1	<0.1	<0.1	1.9 ± 0.8		1.9 ± 1.1	IFP: 6.8 ± 2.8 DMIP: 22.8 ± 12.5 4-MeIQx: <0.1 TMIP: 0.6 ± 0.2	
Thigh, boneless with skin, fried 192 ± 34 °C/4 to 10 min	HPLC-FD-DAD	ng/g	2.37 ± 2.9				0.18 ± 0.2	0.003 ± 0.01	0.11 ± 0.1	IFP: 0.09 ± 0.2	(Salmon and others 2006)
Thigh, boneless with skin, fried 190 ± 37 °C/4 to 10 min, marinated			1.35 ± 1.9				0.75 ± 1.5	0.015 ± 0.03	0.24 ± 0.3	IFP: 0.12 ± 0.3	
Wok, fried/3 min	HPLC-FD	ng/g	1.3								(Ristic and others 2004)

∗ mM Trolox: Antioxidant capacity relative to 6-hidroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid.

Table 16–Content of pyrolytic HAAs in poultry other than chicken.

Sample	Method	Units	Glu-P-2	Glu-P-1	Harman	Norharman	AαC	MeAαC	Trp-P-2	Trp-P-1	Ref.
Goose, oven-broiled, 200 °C/75 min	HPLC-FD	ng/g					0.04 to 0.1	0.1	<0.02 to 0.1	0.2	(Ristic and others 2004)
Pheasant, boiled/60 min							<0.02	0.3	0.6	<0.02	
Turkey, oven-broiled, 180 °C/8 min							<0.02	0.05	0.2	0.1	
Turkey, heated, lyophilized, 180 to 220 °C/10 to 30 min	HPLC-API-ES-MS	ng/g					6 to 16	nq to 13	nq	6 to 56	(Messner and Murkovic 2004)
Turkey, 275 °C/30 min	HPLC-FD-DAD	ng/g fresh wt			1.9	1.3					(Pais and others 1999)
Turkey, breast, pan-fried, 190 °C/12 min	HPLC-FD-DAD	ng/g			12.0	16.5	18.7	nd	2.9	nd	(Brockstedt and Pfau 1998)
Turkey, hot-dog, fried	LC-APCI-MS-MS	ng/g					nd				(Holder and others 1997)

Table 17–Content of thermic HAAs in poultry other than chicken.

Sample	Method	Units	PhIP	IQ	MeIQ	IQx	8-MeIQx	7,8-DiMeIQx	4,8-DiMeIQx	Others	Ref.
Duck, roasted, ready to eat	HPLC-ESI-MS-MS-SRM	ng/g	3.1	<0.1	<0.1		2.4	0.1	0.2		(Richling and others 1998)
Duck, roasted	HPLC-FD-DAD	ng/g	0.46				0.19	nd	<0.1	IFP: nd	(Wong and others 2005)
Duck, stewed			nd				nd	nd	nd	IFP: nd	
Goose, oven-broiled, 200 °C/75 min	HPLC-FD	ng/g	0.9 to 1.0								(Ristic and others 2004)
Pheasant, boiled/60 min			0.6								
Turkey, oven-broiled, 180 °C/8 min			0.8								
Turkey, 275 °C/30 min	HPLC-FD-DAD	ng/g fresh wt	6.8 ± 3.4	<0.1	<0.1	<0.1	1.0 ± 0.5	<0.1	0.19 ± 0.08	IFP: 0.9 ± 0.7 DMIP<0.1 4-MeIQx<0.1	(Pais and others 1999)
Turkey, 275 °C/30 min, meat drippings			4.3 ± 1.6	<0.1	<0.1	<0.1	0.9 ± 0.4	<0.1	1.0 ± 0.5	IFP: 9.6 ± 5.3 DMIP: 6.0 ± 3.1 4-MeIQx<0.1 TMIP: <0.1	
Turkey, heated, lyophilized, 180 to 220 °C/10 to 30 min	HPLC-API-ES-MS	ng/g	24 to 92	nq to 73			nq to 79		nq to 34	DMIP: nq to 12	(Messner and Murkovic 2004)
Turkey, breast, roasted, 160 °C/1 h, well done	HPLC-DAD	ng/g	4.7	nq	2.3		0.9		3.2		(Warzecha and others 2004)
Turkey, breast, pan-fried, 190 to 200 °C/30 min, well done			1.8	nq	nq		9.5		<1.0 ng		
Turkey, breast, pan-fried, 190 °C/12 min	HPLC-FD-DAD	ng/g	64.9				4.4		2.2		(Brockstedt and Pfau 1998)
Turkey, breast, fried, 140 °C/20 min	HPLC-EDC/FD-DAD	ng/g	3.8 ± 1.8	1.1 ± 0.4	0.9 ± 0.3		1.4 ± 0.9		0.4 ± 0.3		(Murkovic and others 1997)
Turkey, breast, fried/cooked	HPLC-UV	ng/g fresh wt	<0.1	<0.1	<0.1		0.6	<0.1	<0.1		(Zimmerli and others 2001)
Turkey, hot-dog, fried	LC-APCI-MS-MS	ng/g	4.4	0.51			4.2				(Holder and others 1997)

and others 2001). The total amount of HAAs showed an increasing trend for each increasing level of MSG. As, the higher the MSG level be the more the consumption of glucose will be in the tissues and the greater the formation of HAAs. In the absence of MSG, the total amount of amino acids decreased, while a reverse trend occurred for the HAAs. Similarly, the higher level of MSG resulted in a higher consumption of amino acids and a larger formation of HAAs. A high correlation was observed between the consumption of taurine, Gly, Ser, or Thr and the formation of HAAs (Tai and others 2001).

Effect of divalent cations. With the addition of iron, the formation of 8-MeIQx increased, while the amounts of harman and norharman decreased, and neither IQx nor PhIP were detected. The addition of Try increased the formation of IQx, 8-MeIQx, harman, and norharman, but the amount of PhIP decreased. Iron or creatinine, together with Try, enhanced the formation of norharman and 8-MeIQx more than when the compounds were added separately (Skog and others 2000). Copper had no enhancing effect on the 8-MeIQx yield (Johansson and Jägerstad 1996). Both cations are catalysts of lipid oxidation and it was suggested that the increase in the 8-MeIQx formation by iron could be due to free radicals formed via iron-catalyzed lipid oxidation. On the other hand, hydroquinone is an initiator of free-radical reactions and does not affect the formation of 8-MeIQx or IQx in the model system, whereas it enhances the amount of 7,8-DiMeIQx. However, when both hydroquinone and $FeSO_4$ were added, a

Table 18–Content of pyrolytic HAAs in eggs.

Sample	Method	Units	Glu-P-2	Glu-P-1	Harman	Norharman	AαC	MeAαC	Trp-P-2	Trp-P-1	Ref.
Fried, 225 °C	HPLC-UV/FD	ng/g			nq	nq			nd, <0.3 ng	nd, <0.3 ng	(Skog and others 1997)
Marinated, with 1% rock candy, without soy sauce	HPLC-DAD	ng/g					<0.21 ng			<0.12 ng	(Lan and Chen 2002)
Marinated, with 1% rock candy and 5% to 20% soy sauce							<0.21 ng			0.14 to 0.52	
Marinated, with 10% soy sauce, without rock candy							<0.21 ng			0.14 ± 0.13	
Marinated, with 10% soy sauce and 0.5% to 5% rock candy							<0.21 ng			0.17 to 0.36	
Marinated, with 10% soy sauce and 1% rock candy, 1 to 4 h	HPLC-DAD	ng/g					<0.21 ng			0.32 to 1.03	(Lan and others 2004)
Marinated, with 10% soy sauce and 1% rock candy, 8 to 32 h							<0.21 ng			1.64 to 3.24	
Marinated, with 10% soy sauce and 1% rock candy, 1 h							<0.21 ng			0.25 ± 0.64	
Marinated, with 10% soy sauce and 1% rock candy, 1 h, with vitamin C							<0.21 ng			0.22 ± 0.38	
Marinated, with 10% soy sauce and 1% rock candy, 1 h, with α-tocopherol							<0.21 ng			0.32 ± 0.58	
Marinated, with 10% soy sauce and 1% rock candy, 1 h, with BHT							<0.21 ng			0.35 ± 1.96	
Egg juice, marinated, with 1% rock candy, without soy sauce	HPLC-DAD	ng/mL					<0.21 ng			<0.12 ng	(Lan and Chen 2002)
Egg juice, marinated, with 1% rock candy and 5% to 20% soy sauce							<0.21 ng			0.63 to 1.04	
Egg juice, marinated, with 10% soy sauce, without rock candy							<0.21 ng			0.15 ± 0.18	
Egg juice, marinated, with 10% soy sauce and 0.5% to 5% rock candy							<0.21 ng			0.44 to 0.75	
Egg juice, marinated, with 10% soy sauce and 1% rock candy, 1 to 4 h	HPLC-DAD	ng/g					<0.21 ng			0.63 to 1.42	(Lan and others 2004)
Egg juice, marinated, with 10% soy sauce and1% rock candy, 8 to 32 h										1.68 to 3.47	

marked increase in these HAAs was observed. Perhaps, the iron oxidizes hydroquinone to a radical that takes part in a free-radical-mediated reaction mechanism (Johansson and Jägerstad 1996). Moreover, Fe^{2+} in the form of myoglobin increased the content of HAAs in meat prior to heating (Murkovic and others 1998).

Effect of oils and antioxidants. It has been reported that the addition of oils and antioxidants may promote or retard the formation of HAAs (Johansson and others 1993; Johansson and Jägerstad 1996; Gu and others 2001). These opposite effects of antioxidants toward formation of HAAs depend on many factors, such as cooking method, cooking conditions, technological factors, and variety and concentrations of antioxidants. These facts make difficult the comparison between *in vivo* and *in vitro* data (Vitaglione and Fogliano 2004). The content of HAAs in raisins may be influenced by grape variety, drying process (sun-drying or mechanical dehydration), sulfur dioxide, drying time, temperature, and moisture (Herraiz 2007). Dark brown raisins are generally produced by allowing grapes to naturally sun-dry on paper trays for 2 or 3 wk, whereas golden raisins (yellow color) are obtained by treating the grapes with sulfur dioxide and usually drying by processes (oven-drying), taking several hours. During the grape-drying process, Try may slowly react in acidic conditions with released aldehydes, mainly acetaldehyde, producing tetrahydro-β-carboline-3-carboxylic acid alkaloids, which are the direct precursors of aromatic β-carbolines. The oxidative conversion of these precursors is accelerated by heating and also in the presence of oxidants and free radicals. In fact, a higher amount of these compounds are found in raisins rather than in the original grapes. Dark brown raisins and raisins obtained by sun-drying processes should contain much higher levels of aromatic-carbolines and tetrahydro-β-carboline-3-carboxylic acids compared to golden raisins or raisins dried following technological dehydration processes and also treated with sulfur dioxide. Sulfur dioxide may also reduce the level of tetrahydro-β-carboline-3-carboxylic acids. Raisin extracts and homogenates exhibited reversible *in vitro* inhibition of monoamino oxidases (MAO) isozymes, particularly MAO-A, suggesting the presence of MAO-inhibiting substances. Two β-carboline alkaloids (norharman and harman) were isolated

Table 19–Content of thermic HAAs in eggs.

Sample	Method	Units	PhIP	IQ	MeIQ	IQx	8-MeIQx	7,8-DiMeIQx	4,8-DiMeIQx	Others	Ref.
Fried, 225 °C	HPLC-UV/FD	ng/g	nd				<2 ng		<4 ng		(Skog and others 1997)
Marinated, with 1% rock candy, without soy sauce	HPLC-DAD	ng/g	<0.03 ng	<0.13 ng	<0.15 ng		<0.30 ng		<0.06 ng		(Lan and Chen 2002)
Marinated, with 1% rock candy and 5% to 20% soy sauce			0.33 to 0.84	<0.13 ng	<0.15 ng		0.32 to 0.53		0.15 to 0.43		
Marinated, with 10% soy sauce, without rock candy			0.34 ± 0.26	<0.13 ng	<0.15 ng		0.33 ± 0.25		0.15 ± 0.08		
Marinated, with 10% soy sauce and 0.5% to 5% rock candy			0.44 to 0.63	<0.13 ng	<0.1 5ng		0.44 to 0.63		0.17 to 0.44		
Marinated, with 10% soy sauce and 1% rock candy, 1 to 4 h	HPLC-DAD	ng/g	0.53 to 1.66	<0.13 ng	<0.15 ng		0.63 to 1.25		0.32 to 1.23		(Lan and others 2004)
Marinated, with 10% soy sauce and 1% rock candy, 8 to 32 h			2.13 to 5.42				2.76 to 4.08		1.85 to 2.85		
Marinated, with 10% soy sauce and 1% rock candy, 1 h			0.46 ± 1.23				0.62 ± 1.08		0.26 ± 0.89		
Marinated, with 10% soy sauce and 1% rock candy, 1 h, with vitamin C			0.32 ± 0.59	<0.13 ng	<0.15 ng		0.68 ± 0.89		0.24 ± 0.43		
Marinated, with 10% soy sauce and 1% rock candy, 1 h, with α-tocopherol			0.38 ± 1.13	<0.13 ng	<0.15 ng		0.52 ± 1.08		0.38 ± 0.76		
Marinated, with 10% soy sauce and 1% rock candy, 1 h, with BHT			0.45 ± 0.83	<0.13 ng	<0.15 ng		0.52 ± 0.47		0.11 ± 0.34		
Egg juice, marinated, with 1% rock candy, without soy sauce	HPLC-DAD	ng/mL	0.03 ± 0.01	<0.13 ng	<0.15 ng		<0.30 ng		0.06 ± 0.02		(Lan and Chen 2002)
Egg juice, marinated, with 1% rock candy and 5% to 20% soy sauce			0.82 to 1.45	<0.13 ng	<0.15 ng		0.63 to 1.25		0.53 to 1.17		
Egg juice, marinated, with 10% soy sauce, without rock candy			0.44 ± 0.18	<0.13 ng	<0.15 ng		0.36 ± 0.25		0.17 ± 0.12		
Egg juice, marinated, with 10% soy sauce and 0.5% to 5% rock candy			0.68 to 0.92	<0.13 ng	<0.15 ng		0.74 to 0.94		0.52 to 0.85		
Egg juice, marinated, with 10% soy sauce and 1% rock candy, 1 to 4 h	HPLC-DAD	ng/g	0.85 to 1.86	<0.13 ng	<0.15 ng		0.94 to 2.18		0.85 to 1.21		(Lan and others 2004)
Egg juice, marinated, with 10% soy sauce and 1% rock candy, 8 to 32 h			2.45 to 5.83				3.26 to 5.62		1.33 to 3.85		

from MAO-inhibiting raisins and accounted for a high percentage of this inhibition, although further studies are required (Herraiz 2007).

Reviews on the role of dietary elements (Chan and others 2005) and on the ability of antioxidants (Vitaglione and Fogliano 2004) to modulate HAA-induced mutagenicity/carcinogenicity have been carried out. Data clearly show a general trend toward a reduction of formation of HAAs both in model systems and in real foods, as well as an effective modulation of biotransformation and metabolism, which implies a decrease in the severity of HAAs-exposure consequences, with some relevant exceptions (Vitaglione and Fogliano 2004). Likewise, contrasting evidence has been reported on the effects of synthetic antioxidants on HAAs-induced mutagenicity and carcinogenicity (Vitaglione and Fogliano 2004). In addition to this case, the comparison among the diverse studies was hampered by the multiplicity of markers observed, by the different experimental conditions, and by the different combination of individual HAAs and antioxidants. Thus, a correlation between the antioxidant properties of the wine and the amount of 8-MeIQx, 4,8-DiMeIQx, and norharman (Busquets and others 2006), as well as with the amount of PhIP and 4′-OH-PhIP (Busquets and others 2007) was found in marinated fried breast chicken. Higher amount of these amines are formed in conditions of shorter marinating time (1 h) and greater antioxidant capacity. On the contrary, long marinades (24 h) with red wine caused a high inhibition of PhIP and 4′-OH-PhIP (Busquets and others 2006, 2007), but also the highest formation of harman (Busquets and others 2006). The reducing effect on PhIP formation in red wine may be related to the presence of some amino acids. However, effects on norharman and PhIP are not due to Try, because this amino acid was not present in the red wines used. Only Pro was found at high concentrations in all the wines tested, and possibly the meat could absorb Pro from the marinating medium, resulting in a reduction of formation of HAAs (Busquets and others 2006).

The amount of PhIP and 8-MeIQx was reduced significantly, around 88% and 40%, respectively, after 6 h of marinating with beer or with wine, but no significant differences were observed with the increase of marinating time. High variations were

Table 20–Content of pyrolytic HAAs in fish.

Sample	Method	Units	Glu-P-2	Glu-P-1	Harman	Norharman	AαC	MeAαC	Trp-P-2	Trp-P-1	Ref.
–	HPLC-ESI-MS-MS-SRM	ng/g	<0.1	<0.1							(Richling and others 1998)
Baltic herring, breaded, fried, 225 °C/4 min	HPLC-UV/FD	ng/g			nq	nq			<0.3 ng	<0.3 ng	(Skog and others 1997)
Baltic herring, breaded, fried, 225 °C/4 min, pan residue					nq	nq			nq	0.03	
Cod fillet, fried, 225 °C/4 min					nq	nq			nq	0.5	
Cod fillet, fried, 225 °C/4 min, pan residue					nq	nq			<0.3 ng	<0.3 ng	
Cod fish, 275 °C/30 min	HPLC-FD-DAD	ng/g fresh wt			5.0	94.8					(Pais and others 1999)
Fish fibre, fried with coconut oil, 120 °C/30 min	HPLC-FD-DAD	ng/g			30.7	51.9	1.8	8.1			(Tai and others 2001)
Fish fibre, fried with soybean oil, 120 °C/30 min					<0.1 ng	5.3	2.2	9.4			
Fish fibre, fried with lard, 120 °C/30 min					13.7 to 16.7	17.0 to 35.2	1.0 to 2.0	1.4 to 7.9			
Fish fibre, fried with lard, 120 °C/30 min, with 9% to 19% of sugars					24.4 to 8.80	41.6 to 10.5	3.1 to 0.9	15.5 to 5.9			
Fish fibre, fried with lard, 120 °C/30 min, with 0.5% to 1.5% of monosodium glutamate					15.3 to 18.4	20.7 to 36.7	1.6 to 5.1	4.5 to 9.7			
Fish fibre, fried with lard, 120 °C/30 min, with 0.01% to 0.1% ascorbic acid					84.7 to 18.8	103 to 40.2	8.3 to <0.1 ng	7.2 to <0.3 ng			
Fish fibre, fried with lard, 120 °C/30 min, with 0.01% to 0.1% tocopherol					9.0 to 15.4	12.4 to 39.6	1.6 to 3.6	5.4 to 1.5			
Fish fibre, fried with lard, 120 °C/30 min, with 0.01% to 0.1% BHT					60.1 to 14.7	107 to 22.0	2.0 to 5.0	9.8 to 1.6			
Hake, raw, frozen	HPLC-FD	ng/g			nd	19.0					(Herraiz 2000a)
Hake, breadcrumb-coated, fried, 10 to 15 min					3.3	13.0					
Hake, grilled, 10 to 15 min					nd	nd					
Hake, grilled, >15 min					8.4	27.0					
Mackerel, fillet, pan roasted, 180 °C/13 to 38 min	HPLC-FD-DAD	ng/g					0.01 to 3.1				(Gu and others 2002)
Mackerel, ordinary muscle, pan roasted, 180 °C/13 to 38 min							<0.001 to 2.8				
Mackerel, dark muscle, pan roasted, 80 °C/13 to 38 min							<0.001 to 0.04				
Mackerel, skin, pan roasted, 180 °C/13 to 38 min							<0.001 to 1.2				
Mackerel, oven-broiled, 200 °C/20 min	HPLC-FD	ng/g					0.1	0.1	0.1	<0.02	(Ristic and others 2004)
Otak-otak, grilled, 3 to 5 min	CZE-UV-DAD	ng/g	0.286 to 1.068		12.8 to 21.3	2 to 13					(Wu and others 1996)
Salmon, barbecued, 270 °C/8 to 24 min	HPLC-FD-DAD	ng/g			3 to 130	8 to 184	2.8 to 109				(Gross and Grüter 1992)
Salmon, oven-cooked, 80 °C/20 to 40 min					<1 to 3	2 to 15	nd				

(*Continued*)

Table 20–(*Continued*)

Sample	Method	Units	Glu-P-2	Glu-P-1	Harman	Norharman	AαC	MeAαC	Trp-P-2	Trp-P-1	Ref.
Salmon, pan-fried, 200 °C/6 to 24 min					2 to 34	8 to 28	nd to 9				
Salmon, fried, 10 to 15 min	HPLC-FD	ng/g			nd	nd					(Herraiz 2000a)
Salmon, fried, >15 min					8.87	6.94					
Salmon, grilled over charcoal, 280 to 300 °C/12 min, medium done: Salmon crust	HPLC-FD-DAD	ng/g	nd	6.6 ± 4.6			8.9 ± 4.8	2.7 ± 2.0	nd	nd	(Costa and others 2009)
"; Salmon fillet			nd	3.18 ± 2.25			3.5 ± 2.4	1.13 ± 0.8	nd	nd	
Salmon, grilled over charcoal, 180 to 200 °C/40 min, medium done: Salmon crust			nd	2.5 ± 1.5			0.93 ± 0.52	nd	nd	nd	
"; Salmon fillet			nd	1.0 ± 0.8			0.37 ± 0.22	nd	nd	nd	
Salmon, grilled on electric griddle, 180 to 200 °C/22 min, medium done: Salmon crust			nd	nd			3.9 ± 2.2	nd	nd	nd	
"; Salmon fillet			nd	nd			1.95 ± 0.1	nd	nd	nd	
Sardine, barbecued, 180 to 200 °C/20 min (medium done) or 280 to 300 °C/10 min (rare done)			nd	nd			nd	nd	nd	nd	
Sardine, barbecued, 280 to 300 °C/12 min, medium done			nd	nd			2.0 ± 0.9	nd	nd	nd	
Sardine, barbecued, 280 to 300 °C/14 min, well done			nd	nd			17.7 ± 2.3	10.6 ± 1.4	8.2 ± 1.1	1.8 ± 0.5	
Swordfish, fried, >15 min	HPLC-FD	ng/g			5.41	40.7					(Herraiz 2000a)
Swordfish, grilled, 10 to 15 min					nd	0.67					

observed for reductions of AαC, ranging between 7% and 77%. Only beer marinade reduced significantly the levels of 4,8-DiMeIQx for 1, 2, and 4 h of marinating (Melo and others 2008b). Red wine presents considerably higher polyphenol contents and antioxidant activity when compared with beer. The effects found in marinated meats can be explained considering other parameters (levels of sugars, alcohol content, time of marinade, and so on), which may promote or retard the formation of HAAs.

The influence of spices has been studied on the formation of HAAs (Murkovic and others 1998). The spices contain antioxidants that can inactivate free radicals (pyrazinium and pyridinium) generated as intermediates during the Maillard reaction (Milic and Milic 1998). Therefore, spices can reduce the formation of HAAs during heating. All the investigated phenolic compounds isolated from common spice plants show strong inhibitory effects on pyrazine cation free radical formation, precursors in the formation of 4,8-DiMeIQx. Also, antimutagenic and anticarcinogenic effects were detected (Milic and Milic 1998). The spices used (Murkovic and others 1998) were rosemary (*Rosmarinus officinalis*), sage (*Salvia officinalis*), thyme (*Thymus* spp), and garlic (*Allium sativum*). As nitrite has antioxidant properties, brine was also applied. The addition of dried spices or brine to the surface of the meat prior to heating leads to a significant reduction in the HAAs content (Murkovic and others 1998). Likewise, rosmarinic acid and a rosemary antioxidant powder decreased the formation of 8-MeIQx and PhIP in beef patties fried at 375 to 400 °C. The effects of the rosemary extracts were more dramatic when cooking temperature and time were increased. However, there was no significant inhibiting effect by any of these extracts on the formation of both harman and norharman (Tsen and others 2006).

Commercial marinades containing spices rich in polyphenolic antioxidants reduced by 57% to 88% the levels of formation of HAAs in grilled steaks (Smith and others 2008). Carnosic acid, carnosol, and rosmarinic acid were the more abundant antioxidants. There were also significant decreases in HAAs for treatments with only the marinade bases, namely, ingredients without any spices/herbs (Smith and others 2008). However, there were no significant differences on HAAs levels of control meat samples (cooked without ingredients) and in meat samples cooked with ingredients usually in the Portuguese diet and rich in antioxidants, such as garlic, wine, olive oil, onion, and tomato (Melo and others 2008a).

The extracts of thyme and Monascus red showed an antioxidative potential. On the contrary, the flavors of marjoram and rosemary showed prooxidative properties. However, thyme, marjoram, rosemary, and Monascus red caused an increase of the concentration of PhIP in a model system (Zöchling and others 2002). By adding flavors of thyme, marjoram, and Monascus red, the increase of PhIP was greater. That is, the content of PhIP was increased regardless of pro- or antioxidative properties. Natural extracts of pine bark (Pycnogenol®), oleoresin rosemary (Herbalox®), and grape seed (Acti Vin™) each at 1% levels, increased the formation of polar and nonpolar HAAs in ground beef cooked at 210 °C for 10 min/side. IQ was not formed in any treatment. Herbalox® decreased the amount of nonpolar HAAs more effectively than

Table 21–Content of thermic HAAs in fish and seafood.

Sample	Method	Units	PhIP	IQ	MeIQ	IQx	8-MeIQx	7,8-DiMeIQx	4,8-DiMeIQx	Others	Ref.
–	HPLC-ESI-MS-MS-SRM	ng/g				nd		nd to 5.3		TriMeIQx: nd	(Richling and others 1998)
Barbecued	GC-NICI-MS-SIM	ng/g	0.5 to 5.5				nd to 0.03		nd		(Tikkanen and others 1993)
Deep-fried	HPLC-FD-DAD	ng/g	0.33				<0.1	nd	<0.1	IFP: nd	(Wong and others 2005)
Pan-fried			0.54				0.32	nd	0.21	IFP: nd	
Stir-fried			nd				<0.1	nd	nd	IFP: nd	
Steamed/boiled, or grilled			nd				nd	nd	nd	IFP: nd	
Baltic herring	HPLC-UV	ng/g	nd	0.2	0.1		0.6		0.3		(Johansson and Jägerstad 1994)
Baltic herring, breaded, fried, 150 to 225 °C/4 min	HPLC-UV/FD	ng/g	0.06 to 0.3				<2 ng to 0.2		<4 ng		(Skog and others 1997)
Baltic herring, breaded, fried, 150 to 225 °C/4 min, pan residue			<0.01				<2 ng to 0.01		<4 ng		
Cod fillet breaded, fried, 150 to 225 °C/4 min			0.02 to 2.2				<2 ng to 0.9		<4 ng		
Cod fillet breaded, fried, 150 to 225 °C/4 min, pan residue			<0.01 to 0.05				<0.01 to 0.2		<4 ng to 0.07		
Cod fish, 275 °C/30 min	HPLC-FD-DAD	ng/g fresh wt	3.2 ± 2.3	<0.1	<0.1	<0.1	<0.1		<0.1	IFP: 2.1 ± 2.0 DMIP: 8.9 ± 6.3 4-MeIQx: <0.1 TMIP: <0.1	(Felton and others 1999; Pais and others 1999)
Cod fish, 275 °C/30 min, meat drippings			18.1 ± 8.5	1.7 ± 0.9	0.7 ± 0.6	<0.1	0.2 ± 0.1		0.04 ± 0.03	IFP: 0.5 ± 0.3 DMIP: 12.8 ± 4.9 4-MeIQx: <0.1 TMIP: <0.1	
Flounder, smoked	HPLC-UV	ng/g	nd	0.7	0.3		1.2		0.6		(Johansson and Jägerstad 1994)
Lemon sole fillet, fried	HPLC-UV	ng/g fresh wt	1.2	<0.1	0.5		<0.1	<0.1	<0.1		(Zimmerli and others 2001)
Mackerel, fillet, pan roasted, 180 °C/13 to 38 min	HPLC-FD-DAD	ng/g	1.6 to 12.8				0.2 to 5.8		<0.1 to 0.4		(Gu and others 2002)
Mackerel, ordinary muscle, pan roasted, 180 °C/13 to 38 min			0.08 to 5.3				0.01 to 2.1		<0.1 to 0.5		
Mackerel, dark muscle, pan roasted, 180 °C/13 to 38 min			0.03 to 1.9				0.01 to 0.9		<0.1 to 0.08		
Mackerel, skin, pan roasted, 180 °C/13 to 38 min			0.3 to 4.2				0.02 to 1.8		<0.1 to 0.2		
Mackerel, oven-broiled, 200 °C/20 min	HPLC-FD	ng/g	1.7								(Ristic and others 2004)
Otak-otak, grilled, 3 to 5 min	CZE-UV-DAD	ng/g	1.6 to 13.0	14.0 to 87.5							(Wu and others 1996)
Salmon, baked, 30 min	CZE-UV-DAD	ng/g	<0.22	<0.20			<0.05		<0.14		(Mardones and others 1998)
Salmon, barbecued, 270 °C/8 to 24 min	HPLC-FD-DAD	ng/g	2 to 73				<1				(Gross and Grüter 1992)
Salmon, oven-cooked, 200 °C/20 to 40 min			nd to 18				<1 to 4.6				
Salmon, smoked	HPLC-UV	ng/g	nd	0.3	nd		1.3		nd		(Johansson and Jägerstad 1994)
Salmon, pan-fried, 200 °C/6 to 24 min	HPLC-FD-DAD	ng/g	1.7 to 23				1.4 to 5				(Gross and Grüter 1992)
Salmon, fried, 150 °C/18 min	HPLC-UV	ng/g	3.0	0.6	1.3		0.6		0.2		(Johansson and Jägerstad 1994)
Salmon, fried, 150 °C/18 min, pan residue			nd	nd	nd		0.5		0.2		
Salmon steak, fried	HPLC-UV	ng/g fresh wt	1.9	<0.1	nd, <0.1		0.5	<0.1	<0.1		(Zimmerli and others 2001)
Salmon, grilled over charcoal, 280 to 300 °C/12 min, medium done: Salmon crust	HPLC-FD-DAD	ng/g	28.9 ± 10	nd	nd	nd	1.0 ± 0.7	nd	nd	TriMeIQx: nd	Costa and others 2009

(*Continued*)

Table 21–(*Continued*)

Sample	Method	Units	PhIP	IQ	MeIQ	IQx	8-MeIQx	7,8-DiMeIQx	4,8-DiMeIQx	Others	Ref.
"; Salmon fillet			13.0 ± 3.3	nd	nd	nd	0.5 ± 0.35	nd	nd	TriMeIQx: nd	
Salmon, grilled over charcoal, 180 to 200 °C/40 min, medium done: Salmon crust			10.6 ± 4.9	nd	nd	nd	3.3 ± 1.2	nd	nd	TriMeIQx: nd	
"; Salmon fillet			4.3 ± 2.0	nd	nd	nd	1.3 ± 0.8	nd	nd	TriMeIQx: nd	
Salmon, grilled on electric griddle, 180 to 200 °C/22 min, medium done: Salmon crust			5.0 ± 0.59	nd	nd	nd	1.7 ± 0.091	nd	nd	TriMeIQx: nd	
"; Salmon fillet			2.6 ± 0.42	nd	nd	nd	0.86 ± 0.09	nd	nd	TriMeIQx: nd	
Sardine, barbecued, 180 to 200 °C/20 min (medium done) or 280 to 300 °C/10 min (rare done)			nd	nd	nd	nd	nd	nd	nd	TriMeIQx: nd	
Sardine, barbecued, 280 to 300 °C/12 min, medium done			3.3 ± 1.0	1.9 ± 0.6	nd	nd	4.4 ± 1.2	nd	nd	TriMeIQx: nd	
Sardine, barbecued, 280 to 300 °C/14 min, well done			6.5 ± 1.3	0.9 ± 0.3	nd	nd	2.2 ± 0.9	nd	nd	TriMeIQx: nd	
Swordfish steak, fried	HPLC-UV	ng/g fresh wt	4.9	<0.1	0.7		<0.1	<0.1	<0.1		(Zimmerli and others 2001)
Seafood, deep-fried	HPLC-FD-DAD	ng/g	0.11				<0.1	nd	<0.1	IFP: nd	(Wong and others 2005)
Stir-fried			0.32				<0.1	nd	<0.1	IFP: nd	
Steamed/boiled			nd				nd	nd	nd	IFP: nd	
Red snapper, whole, with skin, marinated, fried 225 ± 33 °C/5 to 11 min	HPLC-FD-DAD	ng/g	1.37 ± 1.9				0.10 ± 0.3	0.001 ± 0.02	0.03 ± 0.1	IFP: 0.07 ± 0.3	(Salmon and others 2006)
Brown trout, fried 180 °C/8 min, or grilled	HPLC-DAD	ng/g	nd	nd	nd		nd		nd		(Oz and others 2007)
Brown trout, barbecued 20 min			nd	≤0.12	nd		nd		≤0.02		
Rainbow trout, fried 180 °C/8 min, or barbecued 20 min			nd	nd	nd		nd		nd		
Brown trout, grilled			nd	nd	nd		nd		≤0.02		
Seatrout, ready to eat	HPLC-ESI-MS-MS-SRM	ng/g	0.5	<0.1	<0.1		0.1				(Richling and others 1998)

other treatments and, therefore, no AαC and norharman were detected (Ahn and Grun 2005b).

Other studied antioxidants with inhibitory effects are cherry tissue (Britt and others 1998), and soy protein concentrates in marinades (Lan and Chen 2002; Lan and others 2004). Cherry tissue and its methanolic extract inhibited the formation of 8-MeIQx, 4,8-DiMeIQx, and PhIP in pork patties fried at 225 °C for 10 min/side (Shin 2005).

Phenolic compounds, particularly those from tea and olive oil, seem to be the most effective inhibitors of formation of HAAs in model systems, although great variability can be observed because of the concentration-dependent pro- and antioxidant effects. Data have shown that the protection toward action of HAAs depends not only on the quality and concentration of antioxidants, but also on their relative levels in food and on the influence of other minor food constituents on their activity. Some differences have been found among model systems and real foods (Cheng and others 2007). Thus, 12 dietary phenolic compounds were evaluated in relation to their radical scavenging capacities and their inhibition on the formation of HAAs. Four of them, epicatechin gallate (ECG), epigallocatechin (EGC), epigallocatechin gallate, and theaflavin-3,3′-digallate, are tea polyphenols and represent the top antioxidant properties. Carnosic and rosmarinic acids are the principal antioxidants in rosemary. Clorogenic acid, quercetin, quercetin-3-glucoside, and rutin are found in several fruits and vegetables. Naringenin and hesperidin, flavonoids from citrus fruits, are the less active ones as free-radical scavengers. In ground beef patties, except for carnosic acid, all the compounds tested showed significant inhibition of the formation of PhIP, 8-MeIQx, and 4,8-DiMeIQx. But more than 50% of inhibition were achieved when naringenin or theaflavin-3,3′-digallate was added (Table 5). However, in the model systems investigated (Cheng and others 2007), the poor correlation demonstrated between the radical scavenging capacity and their inhibitory activities in the formation of PhIP suggests that antioxidation, more specifically radical scavenging activity, may not be the principal mechanism of intervention of these phytochemicals. Naringenin was also the most active inhibitor in model systems; its capability of simultaneously suppressing the formation of the 3 HAAs studied, and its natural origin suggests a great potential for practical application in daily cuisine (Cheng and others 2007). The opposite effects on the formation of PhIP in model systems and beef patties concerning rosmarinic and carnosic acids are in agreement with other investigations (Zöchling and others 2002; Tsen and others 2006). This also shows that although chemical model systems are, in general, good substitutes for investigating the formation of HAAs, the corroboration in using

Table 22–Content of pyrolytic HAAs in beverages and other foods.

Sample	Method	Units	Glu-P-2	Glu-P-1	Harman	Norharman	AαC	MeAαC	Trp-P-2	Trp-P-1	Ref.
Alcoholic beverages	–	ng/L			1.7	4.8					(Rommelspacher and others 1994)
Beer: Becks											
" : Budweiser					2.4	4.6					
" : Groterjan					0.7	2.7					
" : Guiness					3.2	22.7					
" : Tuborg					1.9	4.9					
" : Weizenbock					5.4	11.7					
Beer	–	ng/L			5.7 ± 3.4	5.3 ± 2.8					(Tsuchiya and others 1996)
Brandy					0.07 ± 0.10	0.11 ± 0.15					
Gin					0.16 ± 0.22	0.12 ± 0.17					
Sake					29.9 ± 42.4	3.6 ± 5.2					
Shochu					0.19 ± 0.26	0.09 ± 0.13					
Vodka					0.17 ± 0.25	0.10 ± 0.14					
Whisky					0.59 ± 0.34	0.41 ± 0.08					
Wine					6.3 ± 5.9	0.62 ± 0.22					
Wines	HPLC-ESI-MS-MS-SRM	ng/L	≤8	nq				≤107			(Richling and others 1997)
Fermented alcoholic beverages	HPLC-FD	μg/L			Σ: nd to 41						(Herraiz 2004)
Coffee extract	HPLC-FD-DAD	μg/g			0.4 ± 0.1	1.3 ± 0.3	nd	nd	nd	nd	(Casal and others 2004)
Coffee, brewed	HPLC-FD	μg/L			Σ: 29 to 207						(Herraiz 2004)
Coffee, brewed espresso	HPLC-FD	μg/L			26.9 to 39.9	91 to 165.6					(Herraiz 2002)
Coffee, ground, and instant		μg/g			0.04 to 1.67	0.09 to 9.34					
Sauces: Soy sauce	–	μg/L			1158 ± 1550	294 ± 86					(Tsuchiya and others 1996)
Tomato ketchup					55.0 ± 35.5	57.9 ± 19.4					
Tabasco					62.1 ± 26.3	21.1 ± 10.5					
Tabasco	HPLC-FD	μg/L			Σ: 4 to 252						(Herraiz 2004)
Vinegar	–	μg/L			110.4 ± 82.7	26.4 ± 12.5					(Tsuchiya and others 1996)
Cocoa		ng/g			161.8 ± 73.1	84.4 ± 38.6					
Cow milk		μg/L			0.46 ± 0.34	0.4 ± 0.08					
Cheese		ng/g			10.7 ± 14.3	3.52 ± 1.22					
"Provola" cheese, traditionally smoked: Core	HPLC-FD	ng/g					13.55 ± 1.79	5.81 ± 1.79	0.45 ± 0.17	2.16 ± 0.92	(Naccari and others 2009)
": Rind							20.45 ± 2.56	10.65 ± 4.99	1.53 ± 0.92	6.33 ± 2.97	
": Slice							18.12 ± 2.71	6.51 ± 2.49	0.92 ± 0.28	4.67 ± 1.80	
"Provola" cheese, buffered smoked							nd	nd	nd	nd	
Offal products: Beef liver, stir-fried, 210 to 225 °C/4 min;	HPLC-ESI-MS-MS-MRM	ng/g			0.30 ± 0.31	1.93 ± 0.43	nd	nd	nd	nd	(Khan and others 2009)
"; and marinated					1.83 to 3.10	1.87 to 8.87	nd	nd	nd	nd	
Beef tongue, fried, 210 to 225 °C/4 min;					0.20 ± 0.17	0.61 ± 0.38	nd	nd	nd	nd	
"; and marinated					0.33 ± 0.20	0.83 ± 0.41	nd	nd	nd	nd	
Lamb kidney, stir-fried, 210 to 225 °C/4 min;					0.55 ± 0.24	0.50 ± 0.10	nd	nd	nd	nd	
"; and marinated					0.32 ± 0.17	0.45 ± 0.13	nd	nd	nd	nd	
Raisins, dark brown	HPLC-DAD-ESI-MS	ng/g			33.4 to 643.5	22.0 to 120.0					(Herraiz 2007)
Raisins, golden					5.5 to 25.0	2.1 to 10.6					
Toasted bread	HPLC-FD	ng/g			Σ: 42 to 160						Herraiz 2004
Bean cake, marinated, with 1% rock candy, without soy sauce	HPLC-DAD	ng/g					<0.21 ng			<0.12 ng	(Lan and Chen 2002)
Bean cake, marinated, with 1% rock candy and 5% to 20% soy sauce							0.23 to 0.62			<0.12 ng	
Bean cake, marinated, with 10% soy sauce, without rock candy							0.22 ± 0.16			<0.12 ng	
Bean cake, marinated, with 10% soy sauce and 0.5% to 5% rock candy							0.33 to 0.55			<0.12 ng	
Bean cake, marinated, with 10% soy sauce and 1% rock candy, 1 to 4 h	HPLC-DAD	ng/g					0.45 to 0.83			<0.12 ng	(Lan and others 2004)
Bean cake, marinated, with 10% soy sauce and 1% rock candy, 8 to 32 h							1.33 to 1.85			<0.12 ng	
Bean cake, marinated, with 10% soy sauce and 1% rock candy, 1 h							0.40 ± 0.98			<0.12 ng	

(*Continued*)

Table 22–(*Continued*)

Sample	Method	Units	Glu-P-2	Glu-P-1	Harman	Norharman	AαC	MeAαC	Trp-P-2	Trp-P-1	Ref.
Bean cake, marinated, with 10% soy sauce and 1% rock candy, 1 h, with ascorbic acid							0.36 ± 1.04			<0.12 ng	
Bean cake, marinated, with 10% soy sauce and 1% rock candy, 1 h, with α-tocopherol							0.41 ± 0.76			<0.12 ng	
Bean cake, marinated, with 10% soy sauce and 1% rock candy, 1 h, with BHT							0.46 ± 0.76			<0.12 ng	
Juice of marinated bean cake, with 1% rock candy, without soy sauce	HPLC-DAD	ng/mL					<0.21ng			<0.12 ng	(Lan and Chen 2002)
Juice of marinated bean cake, with 1% rock candy and 5% to 20% soy sauce							0.71 to 1.83			<0.12 ng	
Juice of marinated bean cake, with 10% soy sauce, without rock candy							0.30 ± 0.28			<0.12 ng	
Juice of marinated bean cake, with 10% soy sauce and 0.5% to 5% rock candy							0.74 to 0.95			<0.12 ng	
Juice of marinated bean cake, with 10% soy sauce and 1% rock candy, 1 to 4 h	HPLC-DAD	ng/g					0.72 to 1.23			<0.12 ng	(Lan and others 2004)
Juice of marinated bean cake, with 10% soy sauce and 1% rock candy, 8 to 32 h							1.44 to 2.50			<0.12 ng	
Smoke condensate from frying of beef, 277 °C/12 min	HPLC-FD-DAD	ng/g					3.48				(Thiebaud and others 1994)

real food systems is critical before inferring the findings (Cheng and others 2007).

Application of tea polyphenols, polyphenon 60® from green tea, and polyphenon B® from black tea to both sides of a hamburger patty before cooking decreased greatly the formation of mutagens in a dose-related fashion (Weisburger and others 2002). The addition of 5% polyphenon 60®, or 597 mg polyphenon B®, led to a reduction from 1065 revertants per plate to 32 and 39.5, respectively (Weisburger and others 2002). Inhibitory effects of green tea catechins (epigallocatechin gallate, EGG), the flavonoids luteolin and quercetin, and caffeic acid on 8-MeIQx and PhIP formation in model systems have been described (Oguri and others 1998). Generation of mutagens was effectively prevented by phenolic antioxidants, sesamol, esculetin, and EGG in a dose-dependent manner (Kato and others 1996). Total content of HAAs in bonito meat was reduced by pretreatment with EGG or green tea extract. The 8-MeIQx content was reduced to 35% of control in a heated chemical model system (Kato and others 1996). Carvacrol, the main ingredient of oregano oil, widely used in salads, was added to ground beef inoculated with *Escherichia coli* (Friedman and others 2009). Carvacrol at the 1% level simultaneously reduced *E. coli* and HAAs. The reduction varied with the temperature of heating (65, 70, and 80 °C) and with the nature of the amine. The patties heated at 70 °C had the greatest reduction (Friedman and others 2009). Flavones and flavonols showed also inhibitory effects that increased in dependence on number and position of hydroxyl functions. A probable mechanism for the inhibition of mutagenesis is the interference by the flavonoids with cytochrome P450 activation of HAAs (Hatch and others 2000). Also, spearmint (*Mentha spicata*) extract inhibited 2 of the major enzymes that play a role in the metabolic activation of IQ, namely, cytochromes P450 1A1 and 1A2 (CYP1A1 and CYP1A2). Consequently, IQ diminished its mutagenic activity (Yu and others 2004).

Studies on ellagic acid and nordihydroguaiaretic acid (NDGA) gave apparently contradictory results. Ellagic acid reduced the formation of 8-MeIQx but enhances that of PhIP, while NDGA had the opposite effect (Oguri and others 1998).

The effects of garlic and several organosulfur compounds on the formation of HAAs and overall mutagenicity were evaluated. PhIP level was reduced up to 81%. The greatest inhibition of total formation of HAAs was achieved with diallyl disulfide (78%) and dipropyl disulfide (70%). These compounds also reduced the overall mutagenicity in a proportional way (Tsai and others 1996; Shin and others 2002a, 2002b). The addition of garlic, diallyl sulfide, allyl methyl sulfide, and allyl mercaptan also reduced the mutagenicity but to a lower extent. However, cystine and cysteine reduced slightly the content of HAAs and, thus, did not reduce the mutagenicity (Tsai and others 1996; Shin and others 2002a, 2002b). Inhibition of IQ-type formation by diallyldisulfide could be mediated through the reduction in the formation of the Maillard reaction products (Tsai and others 1996). A significant inhibition of formation of 8-MeIQx in fried beef patties, up to 70%, was achieved by adding 20 g of garlic to 100 g of marinade. 4,8-DiMeIQx was also reduced by marinating (Gibis 2007). Garlic and lemon juice, as well as honey, were effective in reducing formation of HAAs and overall mutagenicity in marinated meats (Shin and Ustunol 2004). But a higher content of lemon juice in marinades led to only a marginal reduction in 8-MeIQx. The optimum amounts of onion, garlic, and lemon juice that achieved

Table 23–Content of thermic HAAs in beverages and other foods.

Sample	Method	Units	PhIP	IQ	MeIQ	IQx	8-MeIQx	7,8-DiMeIQx	4,8-DiMeIQx	Others	Ref.
Alcoholic beverages: Beer	HPLC-FD	ng/L	30.4 ± 16.4								(Manabe and others 1993c)
Wine			14.1 ± 6.18								
Wines	HPLC-ESI-MS-MS-SRM	ng/L	≤83	≤10	nd		nq			TriMeIQx: nd	(Richling and others 1997)
Coffee extract	HPLC-FD-DAD	μg/g	nd	nd	nd	nd	nd	nd	nd		(Casal and others 2004)
Consommé	GC-NICI-MS	ng/g	<0.2				0.1		<0.1		(Murray and others 1993)
Human milk	HPLC-ES-MS-MS-SRM	ng/L	<0.68								(Scott and others 2007)
"Provola" cheese, traditionally smoked: Core	HPLC-FD	ng/g	3.43 ± 1.77								(Naccari and others 2009)
": Rind			5.46 ± 1.99								
": Slice			5.20 ± 1.98								
"Provola" cheese, buffered smoked			nd								
Offal products: Liver minced, fried/cooked	HPLC-UV	ng/g fresh wt	<0.1	<0.1	1.0		<0.1	<0.1	<0.1		(Zimmerli and others 2001)
Beef liver, stir-fried, 210 to 225 °C/4 min;	HPLC-ESI-MS-MS-MRM	ng/g	nd	nd	nd		nd		nd	DMIP: nd	(Khan and others 2009)
"; and marinated			nd to 0.10	nd	nd		nd		nd	DMIP: nd	
Beef tongue, fried, 210 to 225 °C/4 min;			0.02 ± 0.03	nd	nd		0.21 ± 0.45		0.02 ± 0.04	DMIP: <0.1	
"; and marinated			<0.03	nd	<0.1		<0.04		nd	DMIP: nd	
Lamb kidney, stir-fried, 210 to 225 °C/4 min;			0.11 ± 0.08	nd	nd		0.08 ± 0.04		<0.01	DMIP: 0.25 ± 0.18	
"; and marinated			0.12 ± 0.04	nd	nd		–		nd	DMIP: nd	
Soy-based patties, fried, 226 °C/12 min	HPLC-DAD	ng/g	nd	nd			nd		nd		(Thiebaud and others 1995)
Bean cake, marinated, with 1% rock candy, without soy sauce	HPLC-DAD	ng/g	0.03 ± 0.02	<0.13 ng	<0.15 ng		<0.30 ng		<0.06 ng		(Lan and Chen 2002)
Bean cake, marinated, with 1% rock candy and 5% to 20% soy sauce			0.82 to 1.04	0.13 to 0.43			0.52 to 0.73		1.53 to 1.82		
Bean cake, marinated, with 10% soy sauce, without rock candy			0.81 ± 0.24	0.14 ± 0.28	<0.15 ng		0.52 ± 0.35		0.53 ± 0.18		
Bean cake, marinated, with 10% soy sauce and 0.5% to 5% rock candy			0.93 to 1.45	0.19 to 0.44	<0.15 ng		0.65 to 0.83		0.63 to 1.66		
Bean cake, marinated, with 10% soy sauce and 1% rock candy, 1 h	HPLC-DAD	ng/g	1.12 ± 2.34	0.26 ± 0.78	<0.15 ng		0.68 ± 1.47		0.70 ± .256		(Lan and others 2004)
Bean cake, marinated, with 10% soy sauce and 1% rock candy, 1 to 4 h			1.07 to 2.35	0.32 to 0.66			0.68 to 3.53		1.44 to 1.85		
Bean cake, marinated, with 10% soy sauce and 1% rock candy, 8 to 32 h			3.47 to 6.45	0.85 to 1.96			4.33 to 6.74		2.82 to 3.13		
Bean cake, marinated, with 10% soy sauce and 1% rock candy, 1 h, with ascorbic acid			0.86 ± 1.75	0.24 ± 0.96	<0.15 ng		0.53 ± 2.18		0.76 ± 1.85		
Bean cake, marinated, with 10% soy sauce and 1% rock candy, 1 h, with α-tocopherol			0.98 ± 2.04	0.24 ± 0.58	<0.15 ng		0.76 ± 2.18		0.56 ± 1.19		
Bean cake, marinated, with 10% soy sauce and 1% rock candy, 1 h, with BHT			1.16 ± 2.85	0.24 ± 0.98	<0.15 ng		0.52 ± 0.67		0.68 ± 1.25		
Juice of marinated bean cake, with 1% rock candy, without soy sauce	HPLC-DAD	ng/mL	0.05 ± 0.04	<0.13 ng			<0.30 ng		<0.06 ng		(Lan and Chen 2002)
Juice of marinated bean cake, with 1% rock candy and 5% to 20% soy sauce			1.82 to 2.83	0.53 to 0.74			0.84 to 1.82		1.04 to 2.83		
Juice of marinated bean cake, with 10% soy sauce, without rock candy			0.85 ± .054	0.15 ± 0.18	<0.15 ng		0.63 ± 0.42		0.56 ± 0.63		
Juice of marinated bean cake, with 10% soy sauce and 0.5% to 5% rock candy			1.63 to 1.85	0.53 to 0.75	<0.15 ng		0.86 to 1.55		0.93 to 1.13		
Juice of marinated bean cake, with 10% soy sauce and 1% rock candy, 1 to 4 h	HPLC-DAD	ng/g	1.64 to 3.17	0.63 to 1.05			1.27 to 4.35		1.13 to 1.86		(Lan and others 2004)
Juice of marinated bean cake, with 10% soy sauce and 1% rock candy, 8 to 32 h			4.44 to 6.69	1.33 to 2.14			5.73 to 8.55		2.03 to 3.18		
Smoke condensate from frying of beef, 277 °C/12 min	HPLC-FD-DAD	ng/g	1.47				0.971		0.147		(Thiebaud and others 1994)

a maximum reduction of HAAs were calculated as 31.2%, 28.6%, and 14.6% in marinade (Gibis 2007).

High amounts of ascorbic acid reduced the formation of HAAs, with the exception of harman (Tai and others 2001). In a model system, ascorbic acid in high concentration decreased the 8-MeIQx formation, but not at lower concentrations. The reduction of formation of HAAs by incorporation of vitamin C may be explained by the scavenging of free radicals and oxygen (Johansson and Jägerstad 1996). However, the addition of vitamin C at various levels to pork floss was not effective toward inhibition of HAAs (Liao and others 2009). The addition of a reductone, ascorbate or erythorbate, reduced the mutagenicity of cooked hamburger (Kikugawa and others 2000).

In contrast to the vitamin C, the effect of α-tocopherol on the HAAs in fried fish fiber was concentration dependent (Tai and others 2001). A low amount of α-tocopherol reduced the formation of HAAs more effectively than a high amount. With the exception of MeAαC, the amount of each HAA increased with increasing levels of α-tocopherol (Tai and others 2001). However, in the pork floss, with increasing the levels of vitamin E the HAA contents decreased. The incorporation of 0.1% vitamin E reduced norharman, PhIP, AαC, and MeAαC concentrations (Liao and others 2009). Tocopherols have the ability to inhibit free-radical formation and/or to produce compounds that may react with HAA precursors and prevent their formation.

When vitamin E (1% of fat content) and rosemary oleoresin were added directly to ground beef before frying, a reduction in IQ, MeIQ, 8-MeIQx, DiMeIQx, and PhIP formation was observed. In the same way, the application of vitamin E (1%) to the surface of ground beef patties provided excellent inhibition of formation of HAAs. The oleoresin of rosemary produced a smaller inhibition of PhIP formation than vitamin E. This can be due to the relatively lower concentrations of antioxidant compounds in the oleoresin (Balogh and others 2000).

Carotenoids from tomatoes inhibited the formation of IQx-type HAAs, both in chemical model system (creatinine, glucose, and Gly) and in a freeze-dried bovine meat juice model system. At concentrations of 10 ppm quercetin, the main tomato flavonoid inhibited the 8-MeIQx formation up to 67% (Vitaglione and others 2002).

In a chemical model system, the addition of *tert*-butylhydroquinone (TBHQ), α- or γ-tocopherol, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and propylgallate (PG) increased the amount of 8-MeIQx formed (Johansson and Jägerstad 1996). A slight decrease of PhIP was found in chicken meat in the presence of TBHQ, but no influence on the formation of HAAs was seen in other kinds of meat at any TBHQ concentration added (Messner and Murkovic 2004). However, the introduction of phenolic antioxidants, thiol compounds, BHA, PG, and reductones into a heated model system composed of glucose-Gly-creatinine was effective to scavenge the intermediary pyrazine cation radical and to reduce the mutagenicity due to IQx-type amines (Kato and others 1996; Kikugawa and others 2000). When β-carotene or γ-tocopherol was added to model systems containing hydroquinone and $FeSO_4$, the formation of 8-MeIQx, IQx, and 7,8-DiMeIQx decreased. The free-radical scavengers β-carotene and γ-tocopherol are probably able to scavenge the hydroquinone radicals, thus inhibiting HAAs formation. As it occurs with α-tocopherol, the effect of BHA and BHT on HAAs formation is concentration dependent (Kato and others 1996; Tai and others 2001).

The effects of glycerol, fatty acids, and oils on the yield and species of mutagenic HAAs were studied in a model system, formed by creatinine, glucose, and Gly, heated at 180 °C for 10 or 30 min (Johansson and others 1993). The addition of lipids to the model system did not affect the species of food mutagens formed but affected the yield of 8-MeIQx. The addition of corn oil or olive oil almost doubled the yield of 8-MeIQx formed compared with the amount formed in another model system without fat. This increase was not observed if glycerol or a fatty acid was added to the model system (Johansson and others 1993). The enhancing effect of fats on the yield of HAAs has been attributed to the free radicals produced during thermally induced fat oxidation. However, the degree of oxidation of the fat did not affect the yield of 8-MeIQx.

The addition of virgin olive oil inhibited the formation of IQx derivatives in model systems (Monti and others 2001). The freshness of the oil influenced the antioxidative capacity; the fresher, the better. Fresh oil is richer in *o*-diphenolic compounds. Thus, fresh oil inhibited the formation of IQx, 8-MeIQx, and DiMeIQx by 45%, 50%, and 59%, respectively, whereas 1-y-old oil inhibited the formation by 27%, 13%, and 42%, respectively. This fact supports the hypothesis that formation of HAAs is related to the hydrolysis of dihydroxy phenol derivatives during storage, and this formation partially involves free-radical reactions (Monti and others 2001).

The type of frying oil and the amounts of antioxidants affected the amounts of HAAs formed in beefburgers during frying (Jautz and others 2008). The effect of fat can be misinterpreted by the presence of unsaturated fatty acids, such as oleic and linoleic acids, which are known as inhibitors in the *Ames/Salmonella* test. Frying in virgin olive oil reduced the formation of HAAs compared with frying in refined olive oil (Persson and others 2003a; Jautz and others 2008) and rapeseed oil (Jautz and others 2008). The reduction is probably linked to the high content of phenols in the virgin olive oi1 (Persson and others 2003a; Jautz and others 2008). However, 8-MeIQx seems to form more easily in the presence of phenols. Interestingly, frying in fresh rapeseed oil produced more HAAs than any of the other fresh oil samples, while after 1 y of storage the opposite result was obtained; frying in rapeseed oil produced the lowest amounts of HAAs (Jautz and others 2008). The authors did not have any explanation for this, but rapeseed oil may contain some unknown HAA enhancing components that are lost during storage, or storage may induce the formation of inhibitors. The inhibitory effect decreased during storage, but the addition of antioxidants from rosemary to the oil may counteract this reduction. The addition of rosemary extract to the virgin olive oil resulted in an increase in HAAs formation in the experiment with the fresh oil, but a reduction for the stored oil. The main action of the rosemary extract seems to be in preserving the phenols during storage rather than inhibiting formation of HAAs (Jautz and others 2008).

The influence of 6 frying fats (butter, margarine and margarine fat phase, liquid margarine, rapeseed oil, and sunflower oil) on the formation of HAAs during frying of burgers has been investigated (Johansson and others 1995b). Oxidation status (peroxide and anisidine values), polyunsaturated fatty acids, milk content in the margarine, and antioxidant content (tocopherols/tocotrienols ratio, and vitamin A and E contents) were evaluated before and after frying. The type of frying fat had only minor effects on the formation of HAAs in the beefburgers. However, it influenced the amount of HAAs in the pan residue. The lowest total amount of HAAs was obtained after frying in sunflower oil and margarine.

The observed differences in 8-MeIQx and DiMeIQx formation can be explained in terms of oxidation status and antioxidant content in the frying fat. About half of the content of antioxidants is retained after frying and by liquid margarine. The 2 oils lost their vitamin E activity completely, and the rapeseed oil lost all of its antioxidants. The presence of milk in the liquid margarine reduced its fat oxidation. The results suggest that frying fat rich in antioxidants may reduce the formation of these HAAs during frying (165 to 200 °C). However, the formation of PhIP, which is the most abundant, is not associated with free-radical reactions, and for this reason, it is not affected by free-radical scavenging antioxidants (Johansson and others 1995b). Pan residues from frying beefburgers in butter contained significantly higher amounts of 8-MeIQx and DiMeIQx than samples fried in vegetable oil (Johansson and Jägerstad 1994).

The highly saturated coconut oil may undergo hydrolysis to form a large amount of free fatty acids during heating, which in turn facilitate the degradation rate of lipids and thus promote formation of HAAs (Tai and others 2001). Thus, coconut oil was the most susceptible for HAAs formation in fried fish fiber during heating, followed by lard and soybean oil (Tai and others 2001). Similar results were reported when lard or soybean oils were heated alone. However, no formation of HAAs was detected in the fumes from French fries fried with these oils (Hsu and others 2006). The soybean oil contained vitamin E.

Effects of dietary components on metabolism and health effects of HAAs. Several protective mechanisms from mutagenic and carcinogenic effects of HAAs have been described, and the use of different methodologies for the detection of protective effects has been discussed (Schwab and others 2000). The mechanisms of protection include inactivation of HAAs and their metabolites by direct binding, inhibition of enzymes involved in the metabolic activation of the amines, induction of detoxifying enzymes, and interaction with DNA repair processes. Although much data on HAA-protective compounds have accumulated over the years, only for a few (fibers, pyrroles, constituents of teas, and lactic acid bacteria), there is sufficient evidence to support the assumption that they are protective in humans (Schwab and others 2000).

According to epidemiological studies, diets rich in fruit and vegetables are associated with a low incidence of human cancer. Antimutagenic activities against IQ have been detected in a large number of fruits and vegetables. As the elevation of hydrostatic pressure, in combination with heating, is an alternative to excessive heat in food processing and preservation, homogenates of 14 fruits and vegetable species were exposed to different pressures and temperatures. According to the response of the antimutagenic potential, 3 groups of product could be identified: (1) moderate antimutagenic potencies in grapefruit and strawberries were resistant to both heat and pressure; (2) moderate to strong antimutagenic potencies in carrots, cauliflower, kohlrabi, leek, and spinach were more or less sensitive to heat, but not to pressure; (3) antimutagenic activities of beet and tomatoes were only affected by extreme pressure (Butz and others 1997).

Prolonged and significant reduction in the urinary excretion of 8-MeIQx and PhIP was detected following a 12-d period of high cruciferous vegetable consumption. This reduction in excretion is probably due to an increase in the metabolism of HAAs and suggests that enzyme systems other than CYP1A2 are involved and affected by a cruciferous diet (Murray and others 2001).

Effects of dairy products on HAA-induced rat colon carcinogenesis have been studied (Tavan and others 2002). Four different diets were given to rat groups: supplemented with 20% water, 30% nonfermented milk, 30% *Bifidobacterium animalis* fermented milk, and 30% *Streptococcus thermophilus* fermented milk. The results showed higher inhibition (93% to 96%) of the incidence of aberrant crypts in rats when the diet was supplemented with fermented milk than when the diet was supplemented with milk (66%). There seems to be an early protective effect of milk in the carcinogenesis process in rats (Tavan and others 2002). The urinary excretion of HAAs decreased slightly with simultaneous treatment of male rats with HAAs and lactobacillus (Knize and others 1995).

Summary

A main problem when using literature data related to HAAs contained in cooked dishes is that many experiments have been performed under unspecified cooking conditions, or using high cooking temperatures during a long time to maximize the HAAs production. This last case can lead to a nonrepresentative form of the usual way of cooking meats by the general population in a certain country. The relation between the degree of doneness and the surface browning may differ because some people fry their meats at a high temperature for a short time to get a brown surface but the interior is not so cooked through, while others fry their meats at low temperatures but during a longer time. This can lead to the same degree of surface browning but very different HAAs amounts. In numerous assessments of human exposure to HAAs, photographs showing a variety of surface color have been used to estimate the degree of doneness, the preference of the consumer, and indirectly the content of HAAs (Sinha and Rothman 1997; Keating and others 1999; Keating and Bogen 2001; Alexander and others 2002; Skog 2002). Color development increases with cooking temperature, but it is not possible to estimate the content of HAAs only from color measurements, because no correlation exists with content of HAAs (Solyakov and Skog 2002). In addition, the terminology used for different cooking methods varies around the world. In Argentina and Uruguay, the term "roasting/broiling" does not expose foods directly to flame, whereas "grilling" may use a hot surface (for example, a preheated heavy iron plate), or a grill directly above burning coals (Matos and Brandani 2002; Navarro and others 2004).

Moreover, HAAs belong to a class of numerous compounds and the quantification of each of them in a large number and variety of food samples is not an easy task. Consequently, it is not surprising that bibliographic data are very incomplete (Sanz Alaejos and others 2008a, 2008b, 2008c). Sometimes, food fractions, cooking method, applied temperature, and time of application are not indicated, as shown the Table 2 to 23. The dispersion of the results is evident, and there are scarce data referred to individual HAAs. Even, no data exist for some thermic HAAs, such as 1,5,6-TMIP, 3,5,6-TMIP, 4-CH_2OH-8-MeIQx, and for some pyrolytic ones, Phe-P-1, Orn-P-1, Cre-P-1, and Lys-P-1.

Although recent advances in the analytical instrumentation, concretely in liquid chromatography-mass spectrometry (MS) and gas chromatography-MS (GC-MS) have greatly facilitated the ability to measure HAAs in foods, as well as HAAs and their metabolites in urine of meat eaters, the accurate determination of HAAs is a difficult analytical task, since traces of these compounds have to be determined in highly complex food matrices. This problem can only be solved by combining both elaborate sample preparation steps with selective separation steps, and then followed by sensitive detection methods to quantify low levels of HAAs. Tedious cleanup procedures that include extraction, purification, and preconcentration steps, followed by a separation technique.

Table 24–Some recommendations to minimize the formation of HAAs.

Choose lean cuts. Apply lower temperatures and shorter cooking times.	(Murkovic and Pfannhauser 2000; Salmon and others 2000; Solyakov and Skog 2002)
Grill or pan fry only at low temperature (<180 °C).	(Wild 1996; Zimmerli and others 2001)
Frequent turning of food during cooking.	(Salmon and others 2000)
Direct contact of meat and fish with a naked gas flame or charcoal must be avoided.	(Wakabayashi and others 1992)
Do not allow drippings from meat to become dry before making gravy.	(Zimmerli and others 2001)
Avoid browning of foods or, at least, remove the crust and charred parts of fried or grilled meat, poultry, and fish.	(Wild 1996; Zimmerli and others 2001)
Cooking meat and fish in aluminum foil would reduce charring.	(Wakabayashi and others 1992)
Skin of barbecued chicken must be removed.	(Solyakov and Skog 2002)
Boil or poach fish and stew beef more often, or microwave them.	(Wakabayashi and others 1992; Wild 1996; Zimmerli and others 2001)
Precook meat in an oven or microwave to reduce time on the grill.	
Marinating can significantly reduce the concentration of PhIP in grilled chicken.	(Salmon and others 1997)
For the type of Chinese marinating, both the levels of soy sauce and rock candy must be reduced in order to prevent the formation of HAAs.	(Lan and Chen 2002; Lan and others 2004)
Cooking meat and fish together with foodstuffs containing phenolic antioxidants may be useful to reduce the levels of HAA produced. The same effect may be obtained cooking meat with tomatoes, carrots, or other vegetables rich in carotenoids and antioxidant vitamins.	(Oguri and others 1998)

In addition, the different analytical methods applied might not be comparable. Some problems in determining appropriate estimates of extraction recovery rates must also be taken into account.

Considering that the diverging results can be due to several causes, it should very convenient: (1) to establish databases on HAAs content in cooked foods that are representative for the eating habits of the population being studied, and taking into account each ingredient of the recipe; (2) to record the inside and outside food appearance in the food frequency questionnaires, instead of simply recording the "doneness level;" (3) to consider the possible role of HAAs in the cancer development in conjunction with PAHs and other toxic compounds; and (4) to use biomarkers in order to determine the HAAs exposure.

Although there are not direct indications that HAAs represent a serious health risk to the population, and common cancers are produced by many factors including xenobiotics, all measures to minimize the formation of HAAs should be foreseen. Table 24 shows some recommendations for minimize the formation of HAAs.

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