1 INTRODUCTION

1.1 Introduction

Nowadays cancer is a leading cause of death in human populations all over the world, although the frequencies of tumors in the various individual organs show marked differences among the many countries across the globe. The causes of cancer are understood to be mainly cigarette smoking, infection, chronic inflammation and dietary factors, each of these accounting for up to one third of the total (Sugimura, 2002).

Since one third of cancers are related to food, it is important to look for genotoxic substances or contaminants in foodstuffs. Food can positively contribute to human carcinogens in two ways. The first is related to the presence of genotoxic chemical as food contaminants that induces or increases a change in DNA. The second involves composed of compounds formed by heating food which compounds in this group can indirectly enhance formation of mutagenic compounds and there are the most difficult to remove or avoid completely. (Tribe *et al.*, 2000).

In the mid 1970s, Nagao and coworkers (Tribe *et al.*, 2000) found a high level of mutagen in the charred part of grilled beef, fish and in the smoke produced while broiling sardine. This mutagenicity resulted from compounds were identified as heterocyclic amines (HCAs) and several of these compounds have been classified as carcinogens. Epidemiologist studies have shown a correlation between the intake of fried, broiled or roasted meat and the development of cancer. It has been suggested that heterocyclic amines (HCAs) in cooked foods play a role in the etiology of human cancer. HCAs are formed at part per billion levels during the cooking (Sugimura, 2000). While only present at very low levels, they are very potent mutagens (Lynch *et al.*, 1995).

Heterocyclic amines could be divided into two main classes: the pyrolytic amines (pyrido-indoles and pyrido-imidazoles) and the

aminoimidazo-azaarenes (Jägerstad *et al.*, 1998 cited in Skog *et al.*, 2002). The first group is formed at high temperature, above 300°C, and may be produced by the pyrolysis of amino acids and proteins. The aminoimidazo-azaarenes (imidazo-quinolines, imidazo-quinoxalines and imidazo-pyridines) are created at the ordinary household cooking temperatures of 100-225°C. A pathway of the aminoimidazo-azaarenes is formed from creatine, creatinine, free amino acids, and hexoses produced during the cooking of meat and fish as by-products of the Maillard or browning reaction. The aminoimidazo-azaarenes are sometimes termed thermic mutagens. Since this class of HCAs was found to be extremely mutagenic compared with the other compounds, and more commonly and easily formed during ordinary cooking, they have received more attention over the past decade (Pais *et al.*, 2000).

At present, more than 20 carcinogenic/mutagenic HCAs have been identified and isolated in cooked foods (Wakabayashi *et al.*, 1992; Sugimura, 1997, 2000, Nagao, 1999; Felton *et al.*, 2000 cited in Skog, 2002). Many of these HCAs have been isolated from various protein-rich foods including cooked meat, fish and flavors. In addition, some of these mutagens have been detected in several environmental samples such as outdoor and indoor air, cigarette smoke, cooking fumes, rainwater, incineration ash, diesel exhaust particles (Kataoka, 1997).

Moreover, some HCAs have been detected in biological samples such as urine, plasma, bile and feces. These results suggest that humans are continually exposed to HCAs in a number of ambient environments. On the other hand, some of these mutagenic HCAs have been verified to be carcinogenic to rodents and nonhuman primates and to be implicated in human carcinogenic. Besides, recent investigations revealed that HCAs also possess cardio toxic effect and various pharmaco-toxicological activities such as convulsive activities and potent inhibitory effects on platelet function and dopamine metabolism. Therefore, the isolation and determination of HCAs in

the foods and environment are very important for human health risk assessment (Kataoka *et al.*, 1997).

Selected heterocyclic amines studied in this work were, 2-Amino-3-methylimidazo [4,5-*f*] quinoline (IQ), 2-amino-3,8-dimethylimidazo [4,5-f] quinoxaline (MeIQx) and 2-Amino-1-methyl-6-phenylimidazo[4,5-b] pyridine (PhIP). These three the most biologically active amioazaarenes from cooked meat samples (Janoszka *et al.*, 2001).

2-Amino-3-methylimidazo [4,5-f] quinoline (IQ) was nominated for listing in the Report on Carcinogens by the National Institute of Environmental Health Sciences (NIEHS). Report on Carcinogens (RoC) is based on the review of a 1993 International Agency for Research on Cancer (IARC) monograph which indicated that there is sufficient evidence in experimental animals for the carcinogenicity of IQ and that IQ is probably carcinogenic to humans (Group 2A). Another HCA, PhIP was nominated by Dr. Takashi Sugimura, President Emeritus, and National Cancer Center of Japan. MeIQx was nominated by the National Institute of Environmental Health Sciences for listing in the Report on Carcinogens based on the 1993 IARC evaluation indicating there was sufficient evidence of carcinogenicity in experimental animals for each of these chemicals and that PhIP and MeIQx are possibly carcinogenic to humans (Group 2B) (Technology Planning and Management Corporation through NIEHS, 2002). Body sites in which cancers were found to develop in treated rodents are summarized in Table 1. It is noteworthy that IQ belong to a class of supermutagens, like dinitro- and trinitropyrenes (Sugimura *et al.*, 2000).

Table 1 Carcinogenicities of HCAs in rat and mice

Chemicals	Species	Dose (%) in diet	Target organs
IQ	Rats	0.03	Liver, small and large intestines, Zymbal gland, clitoral gland skin
	Mice	0.03	Liver, fore stomach, lung
MeIQx	Rats	0.04	Liver, Zymbal gland, clitoral gland skin
	Mice	0.06	Liver, lung, hematopoietic system
PhIP	Rats	0.04	Large intestine, mammary gland, prostate
	Mice	0.06	Lymphoid tissue

Source: Sugimura, 2000

These HCAs (IQ, MeIQx, and PhIP) contribute approximately 80% of the mutagenic activity as determined by a laboratory assay (Felton, *et al.*, 1992 cited in www.beefnutrition.org). Of these three compounds, PhIP comprises approximately 83-93% of the combined mass and is the most abundant HCAs in broiled meat, chicken and fish. Representative levels are 0.56 to 69.2 ng g^{-1} cooked food. The level of MeIQx is second highest at 0.64 to 6.44 ng g^{-1} cooked food, and the level of IQ is 0.16 to 0.19 ng g^{-1}. A calculated average intake of carcinogenic HCAs is about 0.4 to 16 mg/day, per capita (Wakabayashi, *et al.*, 1992 cited in www.beefnutrition.org). All humans are

normally and unavoidably exposed to some level of HCAs in foods. Cancer potencies of three heterocyclic amines are showed in Table 2.

Table 2 Cancer potencies of some heterocyclic amines

Compounds	cancer potency/(mg/kg/day)
IQ	26
MeIQx	11
PhIP	3.0

Source: Layton *et al.*, 1995

To assess the risk to human health derived from the daily consumption of foods containing HCAs, an accurate quantification of the amount of potential carcinogens to which man is chronically exposed is essential. Several factors hinder the analysis of HCAs in foods. These analytes are present at part-per-billion levels (ng g^{-1}), which requires an optimization of chromatographic efficiency and both detector highly sensitive and selective analytical methods to be applied. The main objective of this study was to analysis of heterocyclic amines in food by gas chromatography.

1.2 Background

1.2.1 Chemical identification

All of these HCAs share a common imidazole-ring structure with an exocyclic amino group and, therefore, are known chemically as amino-imidazoazaarenes. Most HCAs, including MeIQx and IQ, are fully planar aromatic structures with no bulky out-of-plane functionalities However, PhIP possesses a phenyl moiety that is not necessarily co-planar with the main bicyclic imidazopyridine (Lynch *et al.* 1995) The structures of these HCAs were shown in Figure 1-3.

2-amino-3-methyl-3H-imidazo [4, 5-f] quinoline: IQ

Synonym: 3-methyl-3H-imidazo [4, 5-f] quinolin-2-amine

CAS registry number: 76180-96-6

Molecular formula: $C_{11}H_{10}N_4$

Chemical structure:

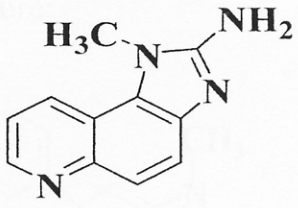

Figure 1 Structure of IQ

2-Amino-1-methyl-6-phenylimidazo [4,5-*b*] pyridine: MeIQx

Synonym: 3, 8-dimethylimidazo [4, 5-f] quinoxalin-2-amine;
2-amino-3, 8-dimethylimidazo [4,5-f] quinoxaline;
Methyl-IQx

CAS registry number: 77500-04-0

Molecular formula: $C_{11}H_{11}N_5$

Chemical structure:

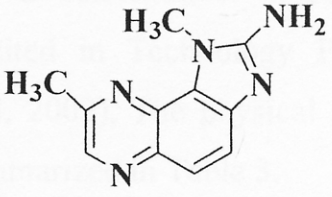

Figure 2 Structure of MeIQx

2-amino-1-methyl-6-phenylimidazo [4, 5-*b*] pyridine: PhIP

Synonyms:1-methyl-6-phenyl-1H-imidazo [4, 5-b] pyridin-2-amine

CAS registry number: 105650-23-5

Molecular formula: $C_{13}H_{12}N_4$

Chemical structure:

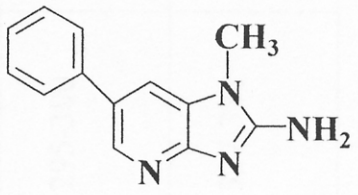

Figure 3 Structure of PhIP

1.2.2 Physical and chemical properties

IQ and MeIQx are stable under moderately acidic and alkaline conditions and in cold, dilute aqueous solutions when protected from light (IARC 1986). They are rapidly degraded by dilute hypochlorite (IARC 1993, 1993c cited in Technology Planning and Management Corporation through NIEHS, 2002), while PhIP is stable under moderately acidic and alkaline conditions (IARC 1993a cited in Technology Planning and Management Corporation through NIEHS, 2002). The physical and chemical properties of IQ, MeIQx and PhIP are summarized in Table 3.

Table 3 Physical properties of IQ, MeIQx and PhIP

Chemicals	Molecular weight ($g\ mol^{-1}$)	Color	Physiscal state	Melting point (°C)	Solubility
IQ	198.22	Light tan	crystalline solid	> 300	• dimethyl sulfoxide • methanol
MeIQx	213.23	Yellow-green	crystalline solid	295-300	• dimethyl sulfoxide • methanol
PhIP	224.26	gray-white	crystalline solid	327-328	• dimethyl sulfoxide • methanol

Source: IARC 1993a, 1993b, 1993c, Knize *et al.,* 1995

1.3 Literature review

1.3.1 Occurrence in food

Many studies have investigated the occurrence of HCAs in food, particularly in meat. IQ is one of many HCAs formed when various meats and fish are cooked. Originally, it was isolated from broiled fish, fried ground beef, and beef extracts. IQ was found when certain compounds were mixed and heated, such as in meat extracts. Meat extracts typically are formed by heating a source of amino acids, reduced sugars, fats, and other ingredients at temperatures greater than 100°C for sufficient times to develop flavor. Aeschbacher *et al.* (1987 cited in Jackson *et al.,* 1994) reported that commercial meat extracts could contain up to 100 ng g^{-1} of HCAs (including IQ, methyl-IQ, and dimethyl-IQ). Creatine and proline heated to 180°C will produce IQ. IQ was also found in mixtures of creatinine and phenylalanine or creatinine, phenylalanine, and glucose, heated to 200°C and in a dry mixture of serine and creatinine heated to 200°C (IARC 1993).

Among the 84 samples of cooked fish, meat, and pan residues Skog *et al.* (1997) found the highest concentration of PhIP (32.0 ng g^{-1}) in the pan residue from a pork fillet cooked at 225°C. A dramatic increase in the formation of HCAs was noted when the cooking temperature increased from 175°C to 200°C; the total amount of HCAs present at 200°C was typically at least twice the concentration detected at 175°C. HCAs formation increased substantially at 225°C, with a notable increase in the pan residues. MeIQx has been detected in some samples of cooked beef, fish, chicken, and mutton; the concentrations ranged from non-detectable to 30 ng g^{-1} (IARC 1993c). Upon heating, MeIQx was formed in concentrated meat juice, and the concentration increased with increasing cooking time and temperature (Arvidsson *et al.,* 1999).

In laboratory tests, PhIP, MeIQ, MeIQx, and DiMeIQx were detected in chicken cooked by different methods. In pan-fried, very well done

boneless, skinless chicken breasts, the levels of PhIP (70 ng g^{-1}) were 2-fold greater than those previously found in very well done pan-fried hamburgers (32.8 ng g^{-1}) (Sinha *et al.,* 1994, 1995). MeIQ was found in small amounts (0.3 ng g^{-1}) in chicken thighs (Pais *et al.,* 1999). Using improved HPLC methods, HCA concentrations were determined in chicken legs that were fried at 200°C for 10 minutes; PhIP (0.21 ng g^{-1}), MeIQ (0.11 ng g^{-1}), and MeIQx (0.13 ng g^{-1}) were all found in these chicken legs (Chen and Yang 1998). Other studies have reported much lower levels of PhIP in chicken. Solyakov and Skog (2002) determined the amount of PhIP and other HCAs in chicken samples that were broiled, deep-fried, pan-fried, oven-roasted, cooked in an unglazed clay pot or in a roasting bag in the oven, and oven-broiled. For 30 chicken samples analyzed, 15 had non-detectable levels of PhIP, while the remaining samples had PhIP levels ranging from trace to 38.2 ng g^{-1}. Literature reviews on HCAs in cooked food have reported typical PhIP levels in chicken of 0 to 40 ng g^{-1} (Solyakov and Skog 2002).

For fish, cooking temperature seems to dictate HCA content as it does in other meats, with higher cooking temperatures producing higher HCA content Gross and Grüter (1992) used HPLC with UV fluorescence detection to determine HCA content in salmon that was prepared and cooked at different temperatures. Salmon was prepared by pan-frying over a gas flame, cooking in a hot-air oven, or barbecuing. The results shown the amount of HCAs in the analysis of restaurant foods and the food in laboratory cooking were agreed well. These studies are a good indicator for U.S. consumption of HCAs in meat. Analysis of HCA content in fast-food products showed undetectable levels of HCAs in 10 of 17 samples. Some studies showed the low levels of PhIP at 0.1 to 0.6 ng g^{-1} in fast-food hamburgers, not detectable to < 0.1 ng g^{-1} in fast-food sausage, chicken, and fish and MeIQx in the range of < 0.1 to 0.3 ng g^{-1} in fast-food hamburgers and not detectable to 0.3 ng g^{-1} in fast-food

sausage, and non detectable levels in fish and chicken samples in fast-food samples (Knize *et al.,* 1995).

The dietary intakes of HCAs are a function of cooking method, doneness preference, and consumption frequency (Keating *et al.* 1999). IQ, MeIQx, and PhIP have been detected in all of the meats commonly consumed by the U.S. population, *i.e.*, beef, pork, chicken, and fish. While very well-done grilled/barbequed chicken produced the greatest levels of PhIP (480 ng g^{-1}) (Sinha *et al.,* 1995). Beefsteaks, hamburger patties, and bacon account for more than 60% of the red meat consumed in the United States (Sinha *et al.,* 2000). Pork, which is the second most frequently consumed meat in the United States, contains very little PhIP. The variation in HCA content of various foods based on cooking temperature, time, and other factors makes the estimation of individual exposure very difficult.

1.3.2 Occurrence in environmental

Environmental occurrence of IQ may arise from food waste and disposal in landfills. IQ is also present in cigarette smoke (Yamashita *et al.,* 1986). Manabe *et al.* (1993) reported the measurement of PhIP in airborne particles, diesel exhaust particles and incineration ash from garbage-burning plants. They proposed that PhIP is likely to be a widespread environmental pollutant and restaurants may release these HCAs into the environment from exhaust emissions and solid or liquid waste disposal. PhIP and MeIQx were found in organic extracts by the blue rayon hanging method from the Yodo River, Japan (Ohe, 1997). The geometric mean in extracts collected from 11 different locations was 11.9 ng g^{-1} (PhIP) and 4.8 ng g^{-1} (MeIQx) blue rayon equivalent. They indicated the possible occurrence of these compounds in the environment. PhIP and MeIQx are found in aerosol droplets that formed during the cooking process, thereby leading to possible inhalation exposure. While increased cooking temperatures has been show

increase HCA production, a concomitant increase in the formation of airborne by-products was also found (Thiébaud *et al.,* 1995).

1.3.3 Biological indices of exposure

IQ undergoes metabolic activation to yield the reactive metabolite *N*-acetyl-IQ, which binds to DNA (Probst *et al.*, 1992). Using the ^{32}P-postlabeling method, Fan *et al.* (1995) found IQ-DNA adducts in human mammary gland epithelial cells following in vitro exposure to IQ. Leong- Morganthaler *et al.* (1998) also found IQ-DNA adducts in TK6 human lymphoblastoid cells following in vitro exposure to IQ, using the same method. Ji *et al.* (1994) studied the urinary excretion of MeIQx among 47 African-American, 41 Asian-American (Chinese or Japanese), and 43 non-Hispanic white male residents of Los Angeles County. Significant interracial differences were observed. Mean levels of MeIQx excretion were 1.3 and 3.0 fold higher in African-Americans than in Asian-Americans and whites, respectively.

Urinary levels of MeIQx were positively associated with intake frequencies of bacon, pork/ham, and sausage/luncheon meats among study subjects. Urinary PhIP metabolites appear to be the most widely used biological markers. The four major urinary PhIP metabolites in humans are *N*2-OH-PhIP*N*2-glucuronide, PhIP-*N*2-glucuronide, 4'-PhIP-sulfate, and *N*2-OH-PhIP-*N*3-glucuronide. Kulp and coworkers (2000) performed a study in which female volunteers who consumed samples of cooked chicken. Urine analysis was performed to quantify the amount of PhIP metabolites excreted. PhIP dose in the urine as PhIP metabolites accounted for 4% to 53% of the ingested dose. The rate of metabolism varied among the volunteers, with the majority (62% to 85%) of the metabolites excreted in the first 12 hours. While urinary PhIP metabolites are a good indicator of recent exposure, endogenous macromolecule adducts could provide more information about long-term exposure. Serum albumin and globin appear to be suitable biomarkers to assess

dietary exposure and internal PhIP doses. In one study, PhIP was found in 12 of 14 human hair samples, with concentrations ranging from 50 to 5,000 pg g^{-1} hair, while two other samples were below the limit of detection (50 pg g^{-1} hair) (Reistad *et al.*, 1999). White/gray hair had an approximately 50% lower PhIP concentration than mixed pigmented, gray, and white hair samples, indicating that melanin participates in PhIP binding. While no dose-response relationship was found and an exposure assessment was not performed, PhIP could be reliably quantified; leading to the possibility that human hair could be used as a biomarker for dietary exposure. HCAs were also found in breast milk of healthy Canadian women (DeBruin *et al.*, 2001). PhIP was detected in 9 of 11 milk samples and the amount were as high as 0.059 ng mL^{-1} (59 ppt). No PhIP was detected in the breast milk of a vegetarian woman, with a limit of detection estimated to be 0.003 ng mL^{-1} (3 ppt).

1.3.4 Sample preparation

Sample preparation techniques are necessary before the identification and quantification of carcinogenic heterocyclic amines from food samples and involve several steps. First is the solution step, this involves protein precipitation by homogenization of the samples with methanol (Edmonds *et al.*, 1986 cited in Pais and Kniz, 2000) or an aqueous acid or basic solution or by enzymatic extraction (Manabe *et al.*, 1991 cited in Pais and Kniz, 2000). In all cases, the sample treatment after the solution step often is a separation technique such as centrifugation or filtration after protein precipitation.

To achieve low detection limits, purification and preconcentration stages are also required. This is commonly carried out by one or various separation procedures including liquid-solid extraction, with XAD-2 resins, blue cotton or rayon. Liquid-liquid extraction from a solid support is done with Kieselgur or Extrelut diatomaceous earth columns. An Ultrasonic

and Soxhlet extraction methods are performed with acetone. The disadvantages of these techniques, were that they required large amounts of solvent which are costly. It must concentrated before determination and the process is single sample run that takes several hours or days to complete. Further purification is carried out with preparative chromatography, column chromatography with Sephadex LH 20 or TSK-Gel CM-650 or solid phase extraction with PRS (propylsulfonic acid), C_{18}, silica or strong cation- exchange (SCX) cartridges (Pais and Knize, 2000).

Recently solid-phase microextraction (SPME) was applied coupled with high-performance liquid chromatography (HPLC) with UV diode array detection (DAD) for the analysis of HCAs. Four kinds of fiber coatings: Carbowax–templated resin (CW–TPR), Carbowax–divinylbenzene (CW–DVB), poly (dimethylsiloxane) divinylbenzene (PDMS–DVB) and polyacrylate (PA) were evaluated for extraction of nine most biologically active heterocyclic aromatic amines (IQ, MeIQx, MeIQ, Norharman, Harman, Trp-P-2, Trp-P-1, AαC and MeAαC) in beef extract. Percentage recovery for each compound is indicated in the range of not recovery to 82.4 %. The advantages of this new method are the reduced amounts of time and organic solvents required (Cárdenes, *et al.*, 2004).

1.3.5 Analysis methods

Several methods have been used for the identification and quantification of HCAs (Kataoka, 1997; Pais and Knize, 2000) on HPLC with UV or fluorescence detection. The limits of detection of the methods were generally in the region of 0.01-50 ng g^{-1}, depending on the compound analyzed and the sample purity. Most of the methods could detect 10-15 HCAs (IQ, MeIQ, MeIQx, 4, 8-DiMeIQx, 7, 8-DiMeIQx, TriMeIQx, PhIP, AαC, Trp-P-1, Trp-P-2, Glu-P-1, Glu-P-2, harman and norharman) in a single run. HPLC in combination with mass spectrometry (LC-MS) was used by Gross *et al.* (1993)

to analyze highly complex extract with atmospheric pressure ionization (Pais *et al.*, 1997; Tribe *et al.*, 2000) and with an electrospray (Pais *et al.*, 1997). MS seems to be a valuable tool in obtaining reliable results in the analysis of complex matrices. There are also reports on the use of Liquid chromatography-tandem mass spectrometry (LC-MS/MS) for the analysis of HCAs (Richling *et al.*, 1998; Guy *et al.*, 2000). LC-MS/MS was used for the quantitative analysis of PhIP released after hydrolysis of PhIP adducted proteins from human blood and in urine; this method of detection is extremely specific and sensitive with a limit of detection of 0.2 fmol PhIP/mg protein (equivalent to 0.045 ng PhIP/g protein) (Magagnotti *et al.*, 2000).

More recently, a liquid chromatography–electrospray mass spectrometric (LC/ES/MS) method for the determination of PhIP, MeIQ, and MeIQx has been developed. This method is more sensitive and more stable than the normally used HPLC/ultraviolet (UV) method. LC/ES/MS also allowed for simultaneous determination of various HCAs in highly complex matrices such as beef extracts and produces chromatograms that are almost free of interferences. In a complex matrix like beef extracts, the detection limit for PhIP was 0.3 ng g^{-1}; for MeIQ, 0.3 ng g^{-1}; and for MeIQx, 1.1 ng g^{-1} (Pais *et al.*, 1997). Richling *et al.* (1997) also have used a similar method of high-performance liquid chromatography-electrospray tandem mass spectrometry (HPLC/ESI/MS/MS) for analysis of heterocyclic amines in wines.

For HCAs, LC is the most appropriate separation method, and direct LC-MS analysis is an effective way to obtain both quantitative and qualitative information. MeIQ was isolated from foods using silica-gel column chromatography and then purifying the sample using reverse-phase HPLC. Later studies used HPLC-thermospray-MS to isolate and extract MeIQ from food samples (IARC 1993b). MS and ultraviolet spectrophotometry identified MeIQx in food using column chromatography and reverse phase HPLC with

analysis. The "blue cotton" adsorption technique, in which trisulfocopper phthalocyanine residues are covalently bound to cellulose or cotton, also has been used extensively in several procedures to detect MeIQx in aqueous solutions. One such procedure quantifies MeIQx using LC thermospray MS. For MeIQx analysis in food extracts, monoclonal antibodies, immobilized on a support for selective immunoaffinity chromotography, were used as a clean-up procedure. MeIQx is also detected in foods using an SPE and medium-pressure LC method. Replicate samples and spiking allow for accurate determination of extraction losses, and a diode array-ultraviolet detector can confirm chromatographic peak identities. Determination of MeIQx levels using these improvements allows for detection limits of 1 ng g^{-1} MeIQx from 3 g of meat or 10 g of fish (IARC 1993c). HCAs have been analyzed by using monoclonal antibodies, either by immunoassay or by purification followed by direct quantification using HPLC-UV (Barnes *et al.*, 1983; Vanderlaan *et al.*, 1990, 1991 cited in Pais and Knize, 2000). Immunoaffinity chromatography has been used to purify IQ and MeIQx from complex mixtures such as heated beef products and beef extracts (Turesky *et al.*, 1989 cited in Pais and Knize, 2000). These methods seem to especially suit for routine analysis of HCAs for which monoclonal antibodies are available. However, due to the complexity of the heated food matrix, monoclonal antibodies have only proven useful in limited applications.

As previously discussed, most of the HPLC-based methods used common UV, fluorescence or electrochemical detection. However, in the last few years, LC-MS has been successfully applied to the determination of HCAs because of its advantage over these conventional detectors: it can provide unambiguous identification. MS is proven to be valuable techniques for the unambiguous identification of contaminants in foods. In recent years capillary electrophoresis (CE) has been widely used for the separation of complex mixtures. The efficiencies of silica capillaries in separation can achieve values

up to two orders of magnitude greater than HPLC columns. Moreover, the simplicity in method development, the rapidity of the analysis, the easy automatic, and the low operation cost, make CE a powerful technique for the analysis of charged solutes. HPLC in combination with electrochemical detection has been used in several studies to analyze HCAs (Galceran *et al.*, 1993). Electrochemical detectors offer increased sensitivity compared with UV detectors, but are limited by the absence of on-line peak confirmation, which is a crucial step in HCAs analysis at the low levels present in cooked foods.

On the other hand, gas chromatography (GC) has been widely utilized for amines analysis because of its inherent advantages of simplicity, high resolving power, high sensitivity and low cost. However, the main problem is the need of derivatzation into less polar compounds because of its strong adsorption. Acetic, trifluoroacetic and heptafluorobutyric anhydrides, pentafluorobenzyl bromide (PFB-Br) and bis-trifluoromethylbenzoyl bromide (bis-TFMBO-Br) have been suggested as derivatization agents for some HCAs. However, acylation with acid anhydrides yielded derivatives with very poor properties for GC analysis. Although the alkylation products with PFB-Br, bis-trifluoromethylbenzyl bromide (bis-TFMBZ-Br) and bis-TFMBO-Cl provided good properties of some HCAs for GC analysis, these methods gave mixture of mono- and di-alkylated forms. Incomplete derivatization lead to non-reproducible results. Consequently, a GC method has not yet been reported. N,N-dimethylformamide dimethyl acetal (DMF-DMA) has been used not only for methyl esterification of carboxylic acid but also for one step derivatization of amino acids into N,N-dimethylaminomethylene methyl esters. The reaction with amino group is based on the Schiff base condensation of primary amines. Therefore, it is considered that each HCA give a single derivative by reaction with DMF-DMA. In addition, a GC with nitrogen-phosphorous selective detector was developed for the determination of HCAs with advantage of high response of these compounds in detector due to the nitrogen atoms in the

structure of the HCAs. The GC method was shown to be very sensitive and highly specific, with detection limits of 0.5-1 pg. For food samples, detection limits were very low: 25 pg g^{-1} in beef samples, 2.5-100 pg mL^{-1} in human urine samples, 0.01-0.2 ng g^{-1} in high temperature cooked meats and 0.03-0.20 ng g^{-1} in grilled chicken. Detection limit using GC-NPD were estimated between 2 and 15 pg of HCAs injected. GC-MS has been described to be the most sensitive technique for HCAs analysis in foods. However, the method was developed with HCAs standards and applicability of the method to food samples has still not been reported. Table 4 summarized the most commonly used methods and detectors for the determination of HCAs in cooked foods.

Table 4 Methods for the identification and quantification of heterocyclic amines (HCAs) in cooked foods

Methods	Detector	Detection limits ($ng\ g^{-1}$)	Advantages	Disadvantages
High Performance Liquid Chromatography	UV-diode array (UV-DAD)	0.02-50	•Peak identity and homogeneity	
	Fluorescence	0.03-3	• High sensitivity	• No peak confirmation, only the less polar HCAs are fluorescent
	Electrochemical (ED)	0.05-2	• Good selectivity and sensitivity	• No peak confirmation, isocratic conditions
	Mass spectrometry (MS)	0.01-2	• High sensitivity and specificity. • Columns with smaller diameter give good separation at low flow rates	
Capillary Electrophoresis	UV, ED, MS	35-50	• High separation efficiency, low operation cost	• Sample preparation with high enrichment
Gas Chromatography	MS	0.01-0.2	• Capillary GC gives high separation efficiency	• Derivatization is usually needed
Enzyme-Linked Immunosorbent assay		1	• Simple	• Monoclonal antibodies are only available for a limited number of HCAs

Source: Pais and Knize, 2000

1.4 Objectives

1. To optimize gas chromatographic conditions for the qualitative and quantitative analysis of HCAs.

2. To study the appropriate sample preparation and analysis of HCAs in food samples