# CHAPTER II

# LITERATURE   REVIEW

## 2.1 Nitrite and Gastric Cancer

A number of human cancers such as stomach and esophageal cancers, may be related to nitrosamines and other nitroso compounds formed from nitrate or nitrite and nitrosable compounds in diet. (Ames, 1983). Gastric cancer mortality was reported to be correlated with nitrate ingestion (Fine et al., 1976). Ingested nitrate is reduced to nitrite by bacteria in the oral cavity (Spiegelhalder, Eisenbrand and Preusmann, 1976). In addition, nitrite can enter the internal environment through three main routes. First, it is used as a direct food additive, primarily for curing meats products; for suppressing outgrowth of Clostridium botulinum; to fix the color of the product; and to stabilize flavour (Greenberg, 1972; Bard, 1973; Herring, 1973). A second source of nitrite occurs as a result of reduction of nitrate by plant and microbial enzymes (Payne, 1973; Wolff and Wasserman, 1972). Many plants, such as leafy vegetables or certain roots, accumulate extremely high concentrations of nitrate under favorable conditions of soil and water (Phillips, 1968; Heisler et al., 1973). Reduction of nitrate to nitrite is carried out by a large variety of microorganisms naturally present in foods, intestinal contents, sewage and saliva (Tannenbaum et al., 1974). Last, nitrite can also be formed through combustion processes, in which oxides of nitrogen (known atmospheric contaminants) occur in locally high concentrations as in the air of open-flame heated spray drier, and this can lead in turn to low (<5 ppm) concentrations of nitrite in dried products.

### 2.1.1 Nitrite as a Convertor for Direct Mutagen

Various foodstuffs containing the precursors of mutagen which could become mutagenic after treatment with nitrite have been examined. Many investigators (Marqauard, Rufino and Weisburger, 1977; Piacek-Llanes and Tannenbaum, 1982; Tomita, Nakamura and Takenaka, 1982; Wakabayashi *et al.*, 1984) have suggested that direct-acting mutagens/carcinogens formed from nitrite and the precursors of mutagens in the acid conditions of the stomach are possible candidates for the causation of human gastric cancer. For example, fava beans commonly eaten in Colombia where stomach cancer incidence was highly shown direct-acting mutagenicity towards *Salmonella* strains after nitrite treatment (Piacek-Llanes and Tannenbaum, 1982).

Japan also has a high incidence of stomach cancer which may related to dietary habit. Japanese fish, soy sauce, bean paste, fish sauce and Chinese cabbage, which are favorites of the Japanese showed direct acting mutagenicity on *S. typhimurium* TA 100 after nitrite treatment. Wakabayashi *et al.* (1984) have made extensive studies on the appearance of direct-acting mutagenicity of various foodstuffs produced in Japan and Southeast Asia on nitrite treatment. After nitrite treatment, various kinds of pickled vegetable and sun-dried fishes produced in Japan showed direct-acting mutagenicity on *Salmonella typhimurium* TA 100, inducing 1,900-18,000 revertants/g. Salted pickled cabbage eaten by the Korean three times a day contained high levels of total N-nitroso compounds (1,173 $\mu$g/kg) after treatment with nitrite under simulated human stomach conditions. Thus, salted pickled cabbage may play an important role in the gastric cancer in Korea (Seel, *et al.*, 1994) Additionally, the extracts of raw and pickled vegetables and fruits, namely garlic, cabbage, shallot, mushroom, cucumber, ginger, Chinese mustard, bamboo shoot and

mango were treated with nitrite in the absence of metabolic activation. All of them exhibited direct-acting mutagenicity towards both *S. typhimurium* tester strains (Hankimhun, 1997).

Wakabayashi *et al.* (1983) found that soy sauce showed direct-acting mutagenicity on *S. typhimurium* TA 100 after nitrite treatment. Lin, Wang and Yeh (1979) investigated the mutagenicity of soy sauce and found that in the *Salmonella* microsome test soy sauce treated with nitrite in the range 1,000 to 10,000 ppm was mutagenic in direct relation to nitrite concentration. However, soy bean showed no mutagenicity with nitrite treatment, the nitrosatable precursors in soy sauce must be formed during the fermentation process.

Surprisingly, a dish composed mainly of carbohydrate was also turned to be direct mutagen. Hayatsu and Hayatsu (1989) treated an aqueous homogenate of boiled rice with nitrous acid at pH 3 and found that mutagens were formed. The mutagen was active in *S. typhimurium* TA 100 and TA 98 without metabolic activation.

A commonly used food additive, sorbic acid could react with nitrite to yield mutagens (Namiki *et al.*, 1981). The same workers also tested the mutagenicity of spices after treatment with nitrite (Namiki *et al.*, 1984). Among severals spices treated with nitrite, pepper exhibited the strongest mutagenic activity in the Ames test, while nutmeg, chili pepper, and laurel also showed strong activities. Shenoy and Choughuley (1992) studied mutagenic products from the nitrosation of piperine. They found some unidentified mutagenic products after the reaction with nitrite. No mutagenicity was observed for spices alone. In addition, Shephard, Wakabayashi and Nagao (1993) reported that aspartame (artificial sweetener) which was nitrosated for

10-30 min with 40 mM nitrite (pH 3.5, 37 °C), showed mutagenic activity on *S. typhimurium* TA 100.

Ohshima *et al.* (1989) screened European food items to identify those that showed direct acting genotoxicity after nitrosation *in vitro*. They found that nitrosation of smoked foods, the frequent consumption of which has been associated with an increased risk of stomach cancer, leads to such direct acting genotoxicity. Additionally, Munzner and Wever (1984) found that the products formed by the reaction of beef extract with nitrite were mutagenicity on strain TA 1538, TA 98 and TA 100. The test material exhibited mutagenic activity towards all tester strains with and without metabolic activation.

Compounds contain no nitrogen atom such as polycyclic aromatic hydrocarbon (PAH) were also converted to be direct mutagens. The nitrite treated products of polycyclic aromatic hydrocarbon (PAH) extracts from smoked fish, skin of fresh water catfish, charcoal-broiled chicken wing, rice pork sausage, pork (medium fat) were mutagenicity towards both strains TA 98 and TA 100 (Kangsadalampai, Butryee and Manoonphol 1996). New substances were not N-nitroso compound but possible were nitro-PAHs. In addition, Peerawong and Kangsadalampai (1998) found that commercial chicken extracts in the presence of nitrite showed the mutagenicity towards *Salmonella typhimurium* strain TA 98 and TA 100 in the absence of metabolic activation.

Furthermore, the products formed by reacting with nitrite under gastric simulating condition (pH 3-3.5) of *Fomes japonica*, *Ganoderma applanatum*, Hed-Cha-Jean were mutagenic to both strains TA 98 and TA 100, while Royal jelly was mutagenic to only strains TA 98 (Katipagdeetham, 1996). Mutagenicity of the extracts of Thai medicinal plants after nitrite treatment were found in *Andrographis*

*paniculata* ness, *Carthamus tinctorius* Linn., *Cassia angustifolia* Vahl, *Cassia fistula* Linn., *Centella asiatica* (Linn.) urban, *Curcuma domestica* Val., *Curcuma zedoaria* Rosc., *Cyperus rotumdus* Linn., *Oroxylum indicum* Vent. and *Zingiber officinale* Roscoe. were mutagenic after treated with nitrite (Kangsadalampai, Kusamran and Butryee, 1995).

Formation of mutagenic N-nitroso compound was able to occurred from treatment vegetable extracts with nitrite. Tiedink *et al.* (1988) found that glucosinolates (detected only in cruciferous vegetables) were probably involved in the formation of N-nitroso compounds in certain nitrite-treated vegetables.

Natural flavour occcured during cooking such as 2-acetylpyrrole was isolated as a major flavour component of many foods (Frattini *et al.*, 1977; Furia and Bellanca, 1975). It is a product of various model of maillard browning reaction. The methylene chloride extract of them after reaction with nitrite in buffer solution (pH 3) at 50 °C for 24 hr showed strong mutagenicity to all tester strains, both in the presence and absence of metabolic activation (Yen and Lee, 1986).

Finally, drug treated with nitrite was to be considered. The interaction of oral administered with nitrite under mildly acidic conditions was considered from a safety point of view of the drugs. Common drugs including aminopyrene (tertiary amines) react with nitrite to form dimethylnitrosamine (or diakylnitrosamines) (Lijinsky, Conrad and Van de Bogart, 1972; Lijinsky, 1974). Several amine drugs were tested as to whether they can produce nitrosamines, N-nitroso and C-nitroso compounds (Rao and Kristina, 1975; Andrews , Fornwald and Lijinsky, 1980; Lijinsky, Reuber and Blackwell, 1980; Takeda and Kanaya, 1981; Takeda and Kanaya, 1982; Andrews, Lijinsky and Synder, 1984; Kangsadalampai and Suharitamrong, 1996). Phenolic drugs including bamethane, acetaminophen and etilefrin also become mutagenic on

treatment with nitrite under mildly acidic conditions. It was noted that after

nitrosation, those drugs produced strong mutagenic diazo-compound (Kikugawa, Kato

and Takeda, 1987; Ohta *et al.*, 1988; Kikugawa, Kato and Takeda, 1989).

Therefore, several nitrosatable mutagen precursors in foods taken by people in

high risk areas might be the etiological factor of gastric cancer, investigation must be

continued to elucidate whether nitrosatable compounds are involved in the

development of human cancer, particularly of the stomach.

### 2.1.2 Mutagenicity of Nitrite Containing Foods

### 2.1.2.1 N-Nitroso Compounds

Mirvish (1975) reported that nitrite reacted with secondary amines or amides

and produced mutagenic and carcinogenic N-nitroso compounds under the acidic

conditions in the stomach. N-nitroso compounds are divided into the nitrosamines,

derived from dialkly, alkaryl, diaryl, or cyclic secondary amines; and the

nitrosamides, derived from N-alkylureas, N-alkylcarbamates and simple N-

alkylamides. Most tested nitrosamines and nitrosamides were proved to be strong

carcinogens (Mirvish, 1975). N-nitrosamines required metabolic activation via

microsomal enzymes before expressing their mutagenicity in the *Salmonella*

mutagenesis assay. In contrast, N-nitrosamides are direct-acting mutagens which

expressed their mutagenicity at the site of formation.

Therefore, direct-acting mutagens and carcinogens, which could be formed

from nitrite and food components under the acidic conditions in the stomach, are

thought to be possible candidates for causation of human gastric cancer. Direct-acting

N-nitroso compound, N-methyl-N'-nitro-N-nitrosoguanidine, is known to induce

stomach cancer in animals (Sugimura and Fujimura, 1967; Sugimura and Kawachi, 1973).

**Nitrosamide as a Possible Etiologic Agents for Gastric Cancers**

It is likely that food components are chiefly responsible for gastric cancer, since stomach is where food first comes into prolonged contact with the gastrointestinal mucosa. Different carcinogens might be involved depending on the population group. A number of workers have proposed that nitrosamides e.g. nitrosourea are produced in the stomach from amides and nitrite derived from food and act in that organ to induce cancer. Nitrosamides are a subgroup of the N-nitroso compounds, which are generally strong carcinogens and also include the nitrosamines. The nitrosamide hypothesis is possible for five rerasons:

a) Nitrosamide could be produced in the stomach from nitrite and amides. The stomach is preferred in vivo site because amide nitrosation is catalysed by acid, proceeding ten times faster for each one unit drop in pH, without a pH maximum (Mirvish, 1975)

b) Nitrosamides (unlike nitrosamines) are unstable and hence might act on the stomach if they were produced there. Since enzymic activation is probably not involved in nitrosamide action (Magee, Montesano and Preussmann, 1976) Promoters or inhibitors of carcinogenesis could not act by altering the metabolic activation of these carcinogens.

c) Because of their instability, nitrosamides probably do not persist in foods and thus may not be ingested as such

d) Nitrite occurred in human stomach contents

e) When certain nitrosoureas and nitrosocarbamates were administered per oral to rodents, they produced low to moderate incidence of glandular stomach

adenocarcinomas, which resemble human gastric cancer (Ogiu, Nakadate and Odashima, 1975).

## 2.1.2.2 Precursors of Nitrite Derived Mutagens Excluding N-nitroso Compound

### 1. Polyclic Aromatic Hydrocarbons (PAHs)

Polycyclic aromatic hydrocarbons (PAHs) are other group of toxicants capable of reacting with nitrite. They are highly lipophilic chemicals and present ubiquitously in the environments as pollutants or as products of pyrolysis of organic matter (Larsen and Poulsen, 1987). They have long been of concern as a potential human health hazard, since many members of this class are tumor initiators or promotors or tumorigenic and/or mutagenic *in vitro* and *in vivo* (IARC, 1979). Two major sources exist for the occurrence of PAHs in foods. The first is the deposition and uptake of PAHs from polluted air on food crops such as cereals, vegetables, fruits and vegetable oils. The second significant source is the formation and deposition of PAHs on foods during heat processing methods such as roasting, smoking, and grilling. Curing smoke is normally produced from wood by the initial pyrolytic changes of lignin, hemicellulose and cellulose, followed by secondary reaction leading to the formation of PAHs and a variety of different chemical compounds (Larsen and Poulsen, 1987).

Besides PAHs, a group of PAH derivatives namely nitro PAH has come into toxicologist concern. Nitro-PAHs are groups of fused ring aromatic hydrocarbon containing one or more nitro groups covalently linked to cyclic carbon atoms. An examination of the mutagenic and genotoxic properties of the nitro-PAHs appears to be timely in view of the recent recognition that this group of chemicals may well have a widespread distribution in the environment (Burkitt, 1971)

Another groups of nitro compound precursors were highlighted since it was found that the consumption of smoked fish and meat products is associated with an increase risk of stomach cancer. A commercial hickory condensate (HSC) was evaluatd for its tumor-initiating and/or promoting activities in the glandular stomach using short-term methods *in vivo*. The administration of HSC with nitrite generated new substance(s) also induced unscheduled DNA synthesis in the pyloric mucosa (Ohshima *et al.*, 1989).

## 2. Heterocyclic Amines

Simulated meat flavour is frequently added to many processed foods as flavour enhancers. It described as "processed food flavours" are normally produced commercially by exposing raw meat (beef, chicken, pork, turkey etc.) to prolonged heating at higher than cooking temperatures, in a process that is proprietary to the industry (MacLeod and Seyyedain-Ardebili, 1981). In these process a series of heterocyclic amines may be formed, some of which are known to posses mutagenic and tomorigenic activities (Eisenbrand and Tang, 1993; Felton *et al.*, 1992; Munro *et al.*, 1993; Sugimura and Wakabayashi, 1990).

Processed food flavours are produced by heating a complex mixture of different ingredients for several hours. The ingredients commonly used are proteins (e.g. meat, poultry, eggs, dairy products, fish, yeasts); carbohydrates (e.g. cereals, vegetable, fruits, sugar); fats or fatty acids (e.g. fats of animal or vegetable origin); and other ingredients (e.g. herbs, spices and their extracts) (Gry, 1995). Processed food flavours are added to food in variable amounts usually in the range 0.1 % to 0.2 % by weight, and are most frequently used in products such as soups, snacks, gravies, sauces and convenience food. In addition, process food flavours have mutagenicity too. The study process of flavours, process flavour ingredients, bouillon concentrates

and a pan residue may exhibit variable amount of mutagenic heterocyclic amines including polar heterocyclic amines (IQ) and non polar heterocyclic amines (Non IQ) (Solyakov, Skog and Jagerstad, 1999).
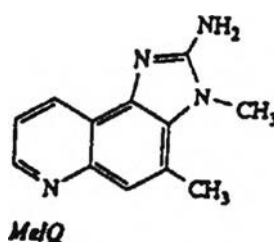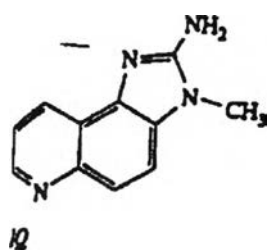
The method of production of food flavours for instant noodle varies and the flavour and aroma of the finished product are mainly dependent on the type of meat, plant materials, spices, and the processing condition used (time and temperature). Most food flavours contained meat (pork, duck, shrimp) and the meat component was thought to be responsible for the formation of heterocyclic amines.

Heterocyclic amines are classified into 2 groups by treatment with 2 mM sodium nitrite. (Robbana-Barnet *et al.*, 1996).
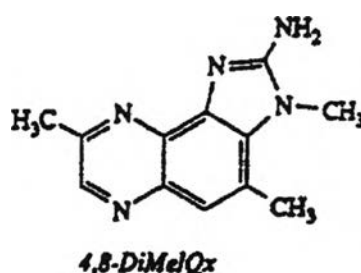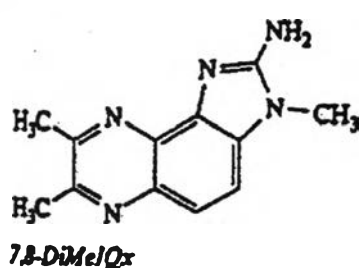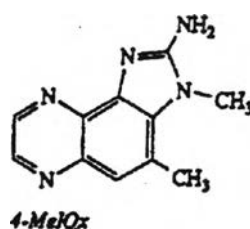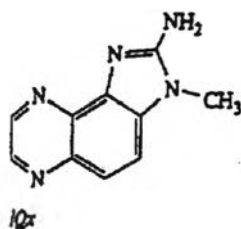
## 1. Aminoimidazoazaarene AIAs (IQ-type, polar)

AIAs compounds have a 2-aminoimidazo group fused to a quinoline (IQ and

Me  )  a    uinoxaline   Me Q  ),  or  a  pyridine  (Ph  )       .    mino  group  is  not

changed by treatment with 2         sodiu     t ite   ut is con  e   ed to a nitro     oup     th

sodium    nitrite.    i   re        sho  s   the   chemical    t    ure    f

inoimid   o       renes    As (    -type, pol   ).

**a            on IQ    e    on po    )**

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include    the    a  inopyridoin  oles    (    -  -       T  -P-2,        C,    MeA  C),    the

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a  ino  henylpyridine    he-P-  )       ino group is conve  ed to a hydro  y   roup   ith 2

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## Quinolines
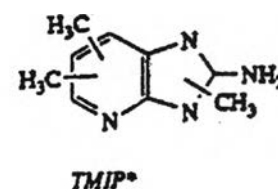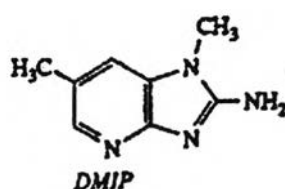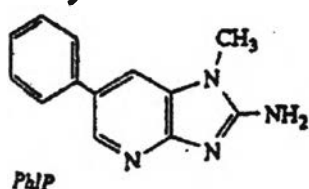


## Quinoxalines



## Pyridines



Figure 1 Chemical structure of Aminoimidazoazaarene AIAs (IQ-type, polar) **Quinolines** (IQ= 2-amino-3-methylimidazo[4,5-*f*]quinoline; MeIQ= 2-amino-dimethylimidazo[4,5-*f*]quinoline); **Quinoxalines** (IQx= 2-amino-3methylimidazo[4,5-*f*]quinoxaline; 4-MeIQx= 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoxaline; MeIQx= 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; 7,8-DiMeIQx=2-amino-3,7,8-trimethylimidazo[4,5-*f*]quinoxaline; 4,8-DiMeIQx=2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline); **Pyridines** (PhIP= 2-amino-1-methyl-6-phenylimidazo[4,5-*b*] pyridine; DMIP= 2-amino-1,6-dimethylimidazopyridine; TMIP= 2-amino-n,n,n-trimethykimidazopyridine )
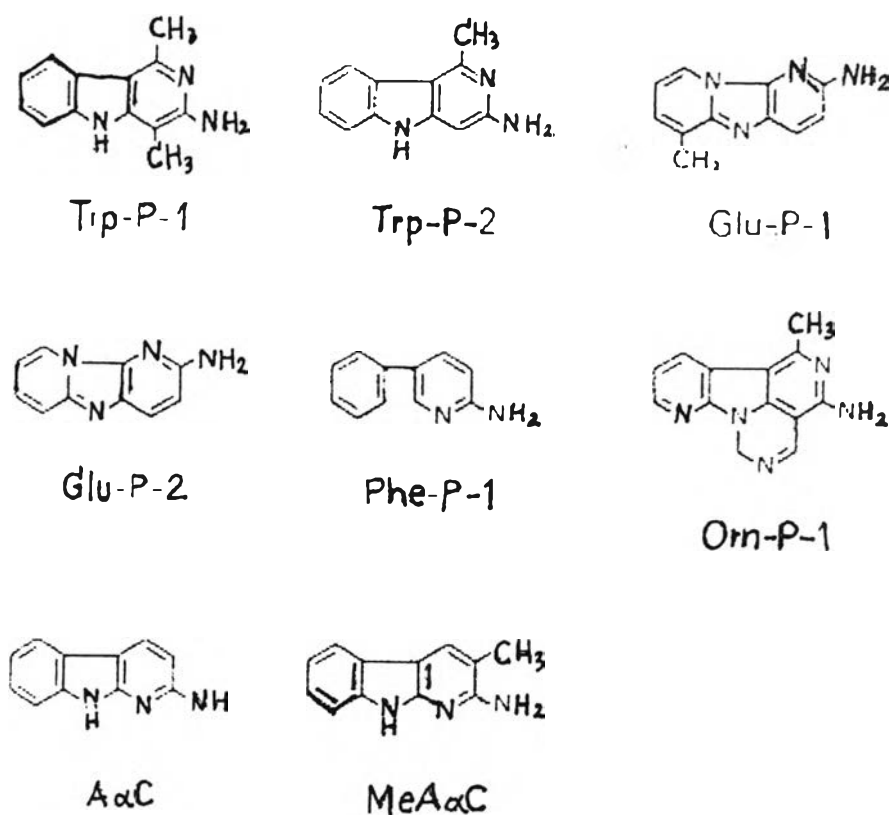
Figure 2 Chemical structure of Carboline (Non-IQ type, non polar) Trp-P-1= 3-3-Amino-1,4-dimethyl-5H-pyrido[4,3-b]indole; Trp-P-2= 3-Amino-1-methyl-5H-pyrido [4,3-b]indole; Glu-P-1= 2-Amino-6-methyldipyrido[1,2a:3,2-d]imidazole; Glu-P-2= 2-Amino-6-methyldipyrido[1,2a:3',2'-d]imidazole; Phe-P-1= 2-Amino-5-phenylpyridine; Orn-P-1= 4-Amino-6-methyl-1H-2,5,10,10b-tetraaza-fluoranthene; A αC= 2-Amino-9H-pyrido[2,3-b]indole; MeAα C= 2-Amino-3-methyl-9H-pyrido [2,3-b]indole

IQ (2-amino-3methylimidazo[4,5-*f*]quinoline) was converted to 3-methyl-2-nitroimidazo[4,5-*f*]quinoline, showing towards *Salmonella* strains without S-9 mix, after 50 mM nitrite treatment at pH 3 (Sasagawa, Muramatsu and Matsushima, 1988) (Figure 3). Formation of a nitro derivative by nitrite treatment was observed with mutagenic and carcinogenic. But, treatment of IQ with a much lower amount of nitrite (2 mM) produced no effect (Tsuda *et al.*, 1985). The other aminoimidazoquinoxaline, aminoimidazoquinoline, aminoimidazopyridine compounds must react with nitrite in a similar manner to IQ. This results suggested that the non-enzymatic formation of direct-acting mutagens from indirect-acting mutagens such as IQ or MeIQ might be physiologically important, especially with regard to the etiology of human gastrointestinal tract tumors (Lin, Cheng and Lin-Shiau, 1992). In addition nitro-IQ induced somatic mutation in *Drosophila melanogaster* (IARC, 1993).
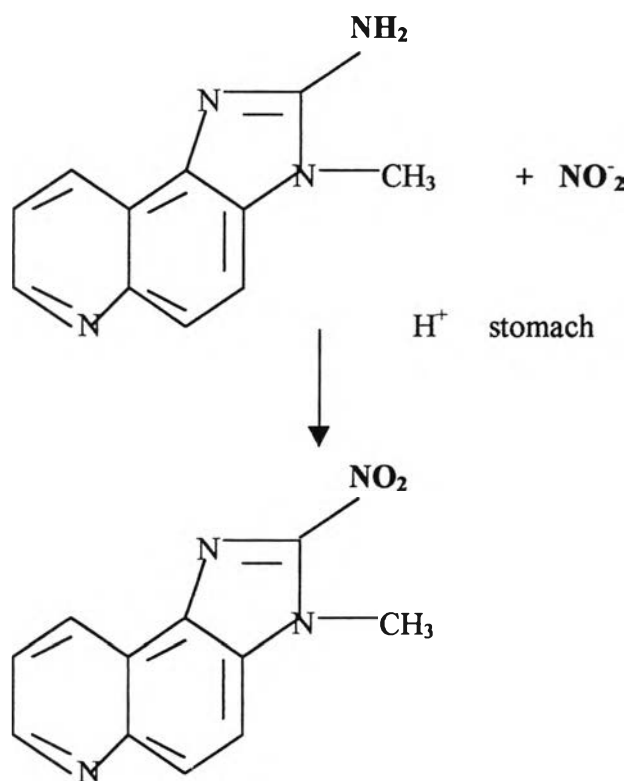


Figure 3   Pathway of formation of nitro-IQ

## Formation of Mutagenic Heterocyclic Amines

Pyrolysing of dry material is performed at high temperatures, usually well above 300 °C. The identification of a new class of mutagenic heterocyclic amines constituting imidazoquinoline (IQ, MeIQ) and imidazoquinoxaline (MeIQx) in the early eighties (Kasai *et al.*, 1980) drew attention to mutagen formation during moderate cooking condition between 100 °C and 300 °C. These food mutagens were isolated from the crust of broiled or fried meat and fish cooked under normal heating condition (100-300 °C), and also from meat extracts manufactured by long term boiling until all the water had evaporated. Non-enzymatic browning (Maillard reaction) was suggested to be involved in their formation (Wei, Kitamura and Shibamoto, 1981; Jagerstad *et al.*, 1983).
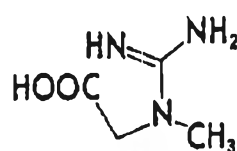
The maillard reaction takes place in foods through the reaction of carbonyl compounds (aldehydes and ketones), notably reducing sugar, such as glucose, fructose, etc., with compounds possessing free amino groups, such as amino acids, peptides, and proteins.

The reaction is of great importance for the development of flavours, texture, and brown pigments during heat treatment of foodstuffs, thereby contributing to the palatability of cooked foods. The number of different compounds that are formed during the reaction is overwhelming. After an initial condensation between reducing sugar and amino groups, rearrangements produce Amadori compounds. These react further by various pathways (enolization, dehydration, retroaldol condensation, Strecker degradation) to produce hundreds of reaction products, viz, furans, pyrazines, imidazoles, pyridines, etc. A tentative pathway for the formation of IQ compounds, by the maillard reaction, was suggested by Jagerstad *et al.* (1983) and it is showed in Figure 4.
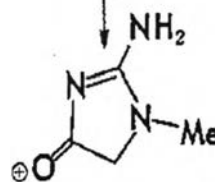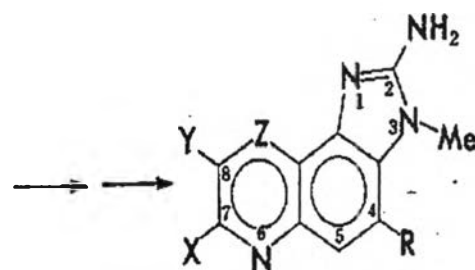
Maillard reaction

$$C_6H_{12}O_6 + RCH \underset{CO_2^{\ominus}}{\overset{NH_3^{\oplus}}{\diagdown}}$$
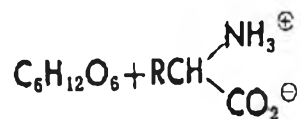
Hexose amino acid

Aldol
condensation

Amadori
rearrangement

Strecker
degradation

$+ RCH \underset{CO_2^{\ominus}}{\overset{NH_3^{\oplus}}{\diagdown}}$

$+ OHC_R$

HOOC, HN=, $NH_2$, N-CH$_3$

Creatine

$NH_2$
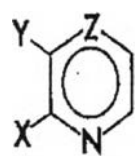
Creatinine

Pyridine
(Z=C)

Pyrazine
(Z=N)

|  | X | Y | Z | R |
|---|---|---|---|---|
| IQ: | H | H | C | H |
| MelQ: | H | Me | C | H |
| MelQx: | H | Me | N | H |
| 7DiMelQx: | Me | Me | N | H |
| 4DiMelQx: | H | Me | N | Me |

Figure 4 Suggested pathway for the formation of imidazoquinolines and

quinoxalines (Jagerstad *et al.*, 1983).

Three precursors, all naturally occurring in muscle meat, were assumed to participate, namely creatine, certain amino acids and sugars. Creatinine was postulated to form the 2-aminoimidazo part by cyclization and water elimination, a reaction that take place spontaneously when the temperature is raised above 100 ° C. The 2-aminoimidazo part is a common moiety of all IQ compounds. It is also responsible for the mutagenicity of the IQ compounds. The quinoline and quinoxaline part of the molecule was postulated to arise through condensation reaction between pyrazines or pyridines and aldehydes.

**Mutagenicity of Heterocyclic Amines**

The most frequently used *in vitro* procedure for mutagenicity test of heterocyclic amines was the Ames *Salmonella*/microsomal assay (Wakabayashi *et al.*, 1992; Felton and Knize, 1991). These compounds show higher mutagenicity in *S. typhimurium* TA 98, a frameshift mutation detector, than in *S. typhimurium* TA 100, a base-pair change mutation detector. Heterocyclic amines are not themselves mutagenic to *Salmonella typhimurium* strains. They need metabolic activation to exert their mutagenicity. They are metabolically activated by cytochrome P450 with conversion of an amino group to a hydroxy group and are further activated into their ultimate reactive forms through N-acetylation and O-acetylation reactions via the enzymes NAT1 and NAT2 (Wakabayashi *et al.*, 1992; Sinha *et al.*, 1994). The principal cytochrome P-4501A2 isozyme responsible for this conversion is induced in rat liver by 3-methylcholanthrene. Hydroxyamino derivatives of heterocyclic amines are further activated by forming esters with acetic acid, sulfuric acid, and proline. These esters may be ultimately form producing DNA adducts. IQ, MeIQ, MeIQx, PhIP, Trp-P-2, Glu-P-1 and MeAαC were reported to form mainly adducts at the C-8

position of the guanine base in DNA. These DNA adducts should be responsible for the genetic alteration leading to carcinogenesis.

All heterocyclic amines tested showed carcinogenic effects in several animal species (Sugimura *et al.*, 1988) including monkeys (Adamson *et al.*, 1990). Furthermore, a weak but positive correlation between the consumption of fried meat products and an elevated risk of colon and other forms of cancer was shown in some epidemiological studies. (Norell *et al.*, 1986; Schiffman and Felton, 1990; Steineck *et al.*, 1990; Willett *et al.*, 1990)


## 2.2 Mutagen and Carcinogens in Wheat Flour

Wheat gluten or flour from several plant sources heated at 210 °C for 1 h produced 0-1800 revertant colonies/g in the Ames/Salmonella test using strain TA 98 with metabolic activation (Knize *et al.*, 1994). A greater mutagenic response in bacterial strain TA 98 than in strain TA 100 and a requirement for metabolic activation suggested that one or more aromatic amine mutagens were formed at normal cooking temperatures, but the mutagenic activity measured could not be account for the known heterocyclic amine commonly found in cooked meat. (Knize *et al.*, 1994). However, wheat and gluten containing products were associated with higher mutagenic activity. Friedman, Wilson and Ziderman (1990) found that extract of heated gluten was highly mutagenic when assayed with *S. typhimurium* TA 98 with metabolic activation. Weak mutagenicity was also observed with strain TA 100 and TA 102. In addition, heated blends of 80 % gluten with 20 % of amylose were mutagenic without metabolic activation.

It was shown that starchy foods preparing by common cooking procedures exhibited significantly mutagenic activity (Spingarn, Slocan and Weisburger, 1980).

Toasting both white bread and dark bread produced the mutagens at the same initial rate but, dark bread produced much higher level of mutagenicity when toasted for long times. They responsed in *S. typhimurium* TA 98 and TA 100 after activation by liver S-9 fraction (Spingarn *et al.*, 1980).


## 2.3 Aminopyrene-Nitrite Mutagenicity Model

Aminopyrene is a natural compound produced in human feces or in anaerobic incubation of 1-nitropyrene with fecal bacterial (Nagahara, Oshita and Nasuno, 1986). 1-Nitropyrene can be metabolized by human, rat and mouse intestinal microflora to 1-aminopyrene and others. The predominant metabolite produced by human, rat and mouse intestinal microflora following 12 h incubation with 1-nitropyrene is aminopyrene.

Aminopyrene was known to be non-mutagenic when it was tested in the Ames assay without metabolic activation (Kinouchi *et al.*, 1982). Kato *et al.* (1991) demonstrated that aminopyrene treated with 4 equivalent amounts of nitrite at pH 3 and 37 ° C showed mutagenicity to *S. typhimurium* strains TA 98 and TA 100 without metabolic activation. The result agreed with the work of Kangsadalampai *et al.* (1996) which stated that nitrite treated aminopyrene exhibited stronger mutagenicity than the authentic aminopyrene towards both strains in the absence of metabolic activation. Thus, the mutagenicity of aminopyrene and nitrite in acid condition has been established as a model for antimutagenicity studies of some chemical concerning the phenomenon occurred during stomach digestion.

## 2.4 The *Salmonella* Mutagenic Assay (Ames test)

In the 1970 Prof. Bruce Ames and colleagues incorporated the latter strategy into the *S. typhimurium* reversion-to-histidine-independence mutagenic assay (Ames, 1972). Mutant colonies are selected by growth on minimal (glucose plus salts) medium, which is supplemented with a trace of histidine, to allow limited growth of histidine-dependent cells and expression of the mutant phenotype. The screening of many available mutant alleles in the *S. typhimurium his* operon allowed identification of ones which are very sensitive to chemically-induced reversion while retaining acceptably low levels of spontaneous mutagenesis. Tester strains were modified by introduction of the deep rough mutation, to archive enhanced cell permeability, and the bacterial DNA excision-repair system (UVR excinucleus) was inactivated, greatly increasing the mutagenic effects of most chemicals. The resulting test system were widely adopted, in the 1970s, for detection of environmental mutagens.

Three of the most important his⁻ alleles found in the Ames tester strains (Hartman *et al.*, 1986) are listed below, along with typical strains bearing the allele; the nature of the mutation in the target gene; and the most common pathway for its reversion:

- *hisD3052*; TA 1538, TA 98: -1 frameshift; ΔGpC frameshift in (GC)$_4$ run

- *hisG46*; TA 1535, TA 100: missense; base-substitution at G:C base-pair

- *hisG428*; TA 102, TA 104, TA 2659: ochre; base-substitution at A:T base-pair

Each Ames test strain evaluates mutagenic activity at a specific (reversion) target sequence. In the case of the frameshift allele *hisD3052* revertants bearing many different sequence changes (spanning a region of more than 50 bp) can be recovered: of course, each such event restores the correct reading frame. Multiple classes of revertants of the base-substitution alleles can also recovered, including transitions,

transversion, and some extragenic suppressor mutations. To emphasize the diversity

of the reversion events found in Ames test alleles, Koch *et al.* (1994) have referred to

them "mini-forward" mutational targets.

An important advance in the development of the Ames test was the

incorporation, in 1975, of plasmid pKM101 (a derivative of a naturally-occurring

bacterial drug- resistant factor), which encodes gene involved in error-prone DNA

repair. The presence of pKM101 enhances the mutagenicities of many mutagens and

in some cases, is absolutely required for mutagenesis (Mc Cann *et al.*, 1975). The

Ames test strains TA 98 and TA 100, while sometimes regarded as a reference set

were constructed independently, and their genetic relationship is neither simple nor

completely understood (Jurado, Alejandre-Duran and Pueyo, 1993). These assay can

be performed in a variety of ways, but the pre-incubation method gave the best result.

Mc Cann and Ames, (1977) discussed several aspects of the experimental basis for

their current assessment of the value of the test as a useful predictive tools :

1. The predictive value of the test as an indicator of carcinogenic potential, including

both the strengths and weakness of the test at this stage in its development

2. Current applications of the test method to problems that were not approachable

using conventional animal test methods.

3. Some of the environmental chemicals that have already been pinpointed as

potential carcinogens by the test and the current status of carcinogenicity tests of these

chemicals in animals.

4. The evidence that the correlation between carcinogenicity and mutagenicity in the

*Salmonella* test reflected more than a useful coincidence and fitted into a compelling

collection of evidence supporting a central role for somatic mutation in the human

cancer.