

R  
008.8  
4467

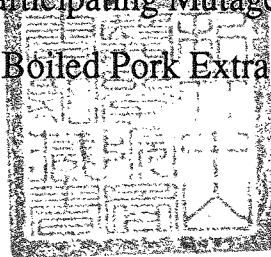
# 中山醫學院醫學研究所碩士論文

Master Thesis , Institute of Medicine ,  
Chung Shan Medical and Dental College

指導教授：李 輝 博士 (Dr. Huei Lee)

長時間煮沸豬肉汁中主要IQ型致突變物的鑑定  
及可能參與形成的化學成份之研究

Identification of Major IQ-type Mutagens and Possible Chemical  
Components Participating Mutagen Formation from  
Boiled Pork Extracts



研究生：林明陽 (Ming-Yang Lin) 撰

中華民國八十二年五月

( May , 1993 )



長時間煮沸豬肉汁中主要IQ型致突變物的鑑定及可能參與形成的化學成份之研究

Identification of Major IQ-type Mutagens and Possible Chemical Components Participating  
Mutagen Formation from Boiled Pork Extracts

本論文為中山醫學院授與理學碩士學位必備條件之一，  
經中山醫學院醫學研究所碩士論文考試委員會審查及口試通過。

論文考試委員

臺灣大學醫學院生物化學研究所

林榮耀 教授

林榮耀

中國醫藥學院環境醫學研究所

郭錦堂 副教授

郭錦堂

中山醫學院醫學系

呂克桓 副教授

呂克桓

中山醫學院生物化學研究所

李 輝 教授

(本論文指導教授)

李 輝

中華民國八十二年六月五日

## 謝 誌

兩年來承蒙恩師 李輝博士在待人處世及研究治學上之教誨與指導，使此一階段的研究工作得以完成。師恩浩瀚，銘記心中，謹誌卷首，以表謝忱！

文稿初成，復蒙國立臺灣大學生物化學研究所林榮耀教授、中國醫藥學院環境醫學研究所郭錦堂副教授及本院醫學系呂克桓副教授等口試委員對本論文的細心審閱並詳加指正，謹此敬表謝意！

研究期間，感謝靜宜大學應用化學研究所所長林孝道教授提供意見並代測質譜，國立中興大學食品科學研究所博士班鄭信男同學協助氣相層析儀的測定，還有本研究室郝菊副教授在研究上的鼓勵並為論文打字提供電腦、邊樹萱老師在實驗技術上的指導及材料的訂購，以及中國醫藥學院詹淑秦講師，汪徽五同學，石名貴、程淑慧、黃明珮、顏慎真、吳政道、關自強、黃玉文、陳淑華、陳怡伶等學弟妹在實驗上的切磋與討論，使本論文得以順利完稿，在此一並致謝。

最後，謹以此論文獻給我的雙親、家人及鈺瑄，感謝他們的關懷與體諒，成為我在研究期間的毅力支柱！

林明陽

謹誌於

中山醫學院醫學研究所

中華民國八十二年六月六日

# 目 錄

## 壹. 文獻綜論

一. 食物加熱後產生的致癌物及致突變物----- 1

二. Maillard反應參與形成食物致突變物----- 7

貳. 中文摘要 ----- 11

參. 緒言 ----- 13

## 肆. 材料與方法

一. 材料與化學藥品 ----- 16

二. 豬肉汁模式系統之建立 ----- 17

三. 致突變性之測定 ----- 17

四. 豬肉汁樣品中致突變物的分析 ----- 18

五. 豬肉汁之化學成份分析 ----- 21

六. 豬肉汁中Maillard反應物的分析 ----- 23

七. 褐變之測定 ----- 25

八. 簡單模式系統樣品中致突變物的分析 ----- 26

## 伍. 結果與討論

一. 豬肉汁萃取物中主要致突變物之鑑定 ----- 28

二. 豬肉汁之游離胺基酸、單醣和肌酸酐在煮沸  
前後的變化及對致突變物形成之影響 ----- 30

三. 豬肉汁中Maillard反應物在煮沸前後的變化和

其添加於豬肉汁形成致突變物的最佳反應條件之研究 .....	34
四。2-Methylpyridine / acetylformaldehyde / creatinine 模式 系統形成IQ致突變物之研究 .....	40
陸。參考文獻 .....	43
柒。圖表 .....	57
捌。英文摘要 .....	106

## 壹。文獻綜論

### 一。食物加熱後產生的致癌物及致突變物

首先在食物中發現的致癌物是Lijinsky and Shubik 在 1964 年由烤肉中偵測到的 pyrene 類致癌物—Benzo[a]pyrene (B[a]P)，接著，Masuda *et al.* (1966) 也在烹煮的魚類及烘培的大麥、焦糖中發現 polycyclic aromatic hydrocarbons (PAH)，Nagao, *et d.* 於 1977 年又報告在菸草濃縮物及表面燒焦的烤魚和牛肉中偵測到致突變性，在另一方面，Sugimura *et al.* (1977) 也由胺基酸(如 tryptophan)熱解物中找到 3-amino-1,4-dimethyl-5H-pyrido[4,3-*b*]indole (Trp-P-1) 及 3-amino-1-methyl-5H-pyrido[4,3-*b*]indole (Trp-P-2) 等致突變物，此時已廣泛引起學者對於研究食物(尤其是肉類食物)加熱後產生致突變物的興趣，然後 Yamamoto *et d.* (1978) 由 *L*-glutamic acid 的熱解物中分離出 2-amino-6-methyldipyrido[1,2-*a*:3',2'-*d*]imidazole (Glu-P-1) 和 2-aminodipyrido[1,2-*a*:3',2'-*d*]imidazole (Glu-P-2) 並確定其結構，Yoshida *et d.* (1978) 也由大豆球蛋白的熱解產物中發現另一種致突變物 amino- $\alpha$ -carboline(A $\alpha$ C)。至此，學者認為肉類食物中的蛋白質、胺基酸等經由高溫(約 500°C-600°C)的熱解後，會產生胺基酸衍生的致突變物，而其主要因素則在於溫度的影響(Matsumoto *et al.*, 1977)。

不過，Commoner *et d.* (1978) 用安氏檢測法測得以一般家庭烹飪方式加熱的牛肉萃取物和煎碎牛肉(漢堡肉)中也會產生致突變物，1980 年 Kaisai *et d.* 也在一般家庭所食用的烤沙丁魚乾中證實產生 IQ 型的異環胺類致突變物—

2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) 及 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoxaline (MeIQ), 這也是最早被發現的 IQ 型致癌物 (Fig.1), 此類化合物的致突變性相當高, 甚至比 aflatoxin B1 還高。另外, Yamaguchi *et al.* (1980b) 和 Yamaizumi *et al.* (1980) 又分別於牛肉及烤魚中發現 Trp-P-1, 而 Matsumoto *et al.* (1981) 也在燒烤食物及香煙濃縮物內找到 AαC, 到此時, 已經發現許多由胺基酸或蛋白質熱解後產生的致突變物, 但只有一例報告提到 IQ 型的異環胺類化合物的發現, 接著, Kasai *et al.* 即在煎牛肉中找到另一類的 IQ 型致突變物— 2-amino-3,8-dimethyl-imidazo[4,5-*f*]quinoxaline (MeIQx) (見 Fig.1)。

至於有關中國傳統調理-"滷"的方式, 是否也會產生致突變性的研究, 一直都無報告提出, Lee *et al.* (1981) 及 Lin *et al.* (1982) 首先報告長時間煮沸豬肉汁中有會造成菌種基因結構轉移的前驅致突變物 (frameshift promutagen), 會引起沙門氏桿菌屬 TA98 加上 S9 混合酵素後產生反突變 (reverse mutation) 菌落, 而影響其形成的因素有肉汁的濃度、酸鹼值及煮沸的時間, 這些促進致突變物形成的影響條件都似乎和加速 Maillard 反應的進行有關, 同時亦指出在長時間煮沸豬肉汁中產生的致突變物和當時已知的食物致突變物— Trp-P-1, Glu-P-1 及 B[a]P 等不同。

Hargraves 和 Pariza (1982) 將市售細菌級的牛肉萃取物、食用的牛肉萃取物和油煎牛肉做一比較, 結果發現細菌級的牛肉萃取物在初步 silicagel 管柱純化時有三個主要波峰區域而在食用的牛肉萃取物及油煎牛肉, 則皆為二個波峰區域, 再經過 LH-20 管柱及高效液相層析  $\text{NH}_2$ 、 $\text{C}_{18}$  等

管柱的純化後，再以質譜儀分析出細菌級的牛肉萃取物中有 MeIQ<sub>x</sub>、MeIQ 和 IQ，而食用的牛肉萃取物所含的致突變物為 MeIQ<sub>x</sub> 和 IQ，至於油煎牛肉，則只確定出含有 MeIQ<sub>x</sub> (Hargraves and Pariza,1982,1983)。還有，Turesky *et al.* (1983) 也定量出細菌級的牛肉萃取物中所含的 IQ 與 MeIQ 分別為 41-142 ng/g 與 142-527 ng/g。另外，Hayatsu *et al.* (1983a,b) 利用具高度純化率的藍棉 (Blue cotton) 將細菌級牛肉萃取物中所含致突變物分析純化，並定量其含率為：MeIQ<sub>x</sub> (222-273 ng/g)，IQ (21-40 ng/g)。而 Barnes *et al.* (1983a) 以薄層色層分析法取代前述的 silica gel 管柱為純化方式，亦由烹調的牛肉及魚肉中找到 IQ 並加以定量，發現在每公斤高脂肪肉類中含 IQ 為 20.1 μg，而每公斤低脂肪肉類中 IQ 的含量為 0.53 μg，由此可見，脂肪含量的高低也會影響到致突變物的形成量。

Felton 等人 (1984) 在煎碎牛肉中又發現另一類新的 IQ 型致突變物— 2-amino-3,4,8-trimethylimidazo[4,5-f] quinoxaline (4,8-DiMeIQ<sub>x</sub>) (Fig.1)，且定出其在牛肉中的含量為 0.06 ng/g，而 Takahashi 等人 (1985a) 也在細菌級牛肉萃取物中找到 4,8-DiMeIQ<sub>x</sub>，其含量為 100 ng/g，此較煎碎牛肉的含量高出 167 倍之多！，根據上述的研究結果似乎可看出，對於各種已被發現的 IQ 型致突變物 (Fig.1) 而言，在細菌級牛肉萃取物中的含量皆遠較一般所食用的煎牛肉含量為高。

Takahashi *et al.* (1985b) 以 "液相層析結合電化學偵測 (LCEC)" 的方法，測定出在牛肉萃取物中所含的致突變物— MeIQ<sub>x</sub> 含量為 3.1 ng/g 食品級牛肉萃取物、58.7 ng/g 細菌級



牛肉萃取物，由於此方法具有較高的靈敏度，因此只需較低量致突變物即可測到，不過其相對干擾也越大。在1986年，Kikugawa 等人在以煙燻的鱈魚乾中測到MeIQx，其含量為2.0 ng/g，由於鱈魚乾的作法是在80-120°C的溫度下加熱50-120小時，因此推測MeIQx的產生是來自魚肉本身的加熱，而並非由於木材燃燒的煙燻所造成。而Kato *et al.* (1986)所提報告也同意這種看法，他們檢測了以各種方式煙燻的肉類食品(如鮭魚、鯡魚、鱒魚、雞肉、火腿、臘腸及鯖魚乾)，結果發現以較高溫(80-140°C)烘乾作法的鯖魚乾具有最高的致突變性，另外以較低溫(50-80°C或15-30°C)加熱和冷、熱煙燻製作的肉類只具極低或無致突變性，所以，不論如何加以煙燻，其致突變性的高低與加熱溫度的關係，幾乎皆成正相關性，接著，取最強致突變性的鯖魚乾予以純化分析出主要致突變物為MeIQx，其含量為0.8 ng/g，而次要的致突變物為4,8-DiMeIQx。

Yamaizumi *et al.* (1986)提出一種適用在食物中致突變物含量約在微克(ppb)範圍的偵測及定量方法，其採用穩定的放射性標定於內置標準品上，再結合液相層析-質譜分析，而準確地分別測定由烘烤鮭魚肉、表皮中產生的致突變物-IQ、MeIQ含量，並且與在烘烤牛肉、沙丁魚中的含量相比較，可看出同樣是以烘烤製作的肉類食物而言，鮭魚的裡肉和表皮的致突變物(IQ、MeIQ)含量低於沙丁魚，但高於牛肉，此意味著不同肉質對致突變物量的影響，而若僅就鮭魚來講，其表皮的致突變性仍略高於裡肉，此即表示鮭魚在烘烤過程中表皮的受熱大於

裡肉，由此也可以看出溫度對致突變物產生的影響。在同時，Grose 等人(1986)也由煎蛋中純化出IQ，不過，其IQ含量(每克煎蛋中只含0.1微克IQ)仍較一般肉類食物含量為低。

Kikugawa *etal.* (1987)又對一些魚類以100°C及220°C分別加熱48及0.25小時後，先測定其初步萃取物的致突變性，結果若以高二倍的溫度加熱時，只需百分之一的時間，即可產生相當的致突變力，而用電熱板加熱(heat-dried)方式所產生的致突變物主要為MeIQx與4,8-DiMeIQx，如以致突變性最強的鱈魚為例，分別所偵測出的含量分別為：52 ng MeIQx/g 與54 ng 4,8-DiMeIQx/g，而以火烤至燒焦狀態時，其所含的致突變物則以IQ、MeIQ或Glu-P-2為主。

另外，關於MeIQx和MeIQx的雙甲基類構物—DiMeIQx，在決定其甲基鍵結位置時，只以紫外線光譜(UV)及質譜(MS)作為分析依據，並無法完全決定出正確的異位結構物，雖然以核磁共振(NMR)分析大多可以區分出其異位結構物，不過所需的樣品量需達10毫克左右，這對於大部分的微量純化過程而言是比較難做到的，因此Knize *et al.* (1987)提出在較少量樣品的情況下，將在樣品中純化得到之致突變物，分別加入二種MeIQx異構物標準品及四種DiMeIQx異構物標準品中，再以高效液相層析合流法(HPLC coelution)配合核磁共振的質子轉移，即可測定出異位結構物上甲基團鍵結的數目及位置，結果以此法在煎碎牛肉中找到8-MeIQx和4,8-DiMeIQx等兩種致突變物。

Zhang 等人(1988)發表研究中國大陸東北地區七種日

常食用的煎、烤一鼓眼狹鱈 (walleye pollack), 斑點鯉魚 (variegated carp), 毛尾 (hairtail) 及羊肉等, 廣泛分析其間致突變性的差異, 此研究不同於多數以研究日本、美國及歐洲等國家人民日常食用肉類的報告, 而欲了解中國大陸東北居民的主要食物和歐、美、日等地人民的日常食品中, 所含致突變物有何異同, 在以各種加熱方式製成的四種肉類食物, 經由萃取出鹼性分層後, 在樣品中加入 S9 混合酵素以沙門氏 TA98 菌種測定其致突變性, 結果在不鏽鋼平底鍋油煎的鼓眼狹鱈具有最强的致突變性, 但在大量蔬菜油中油炸的方式僅得約前法烹調的七十分之一的致突變性, 可見加熱的方式顯著影響致突變性的強度, 而若以同樣方式油煎的斑點鯉魚, 其致突變性只為鼓眼狹鱈的十六分之一, 因此推測此兩種魚類的肉質組成可能有相當程度的差異, 以致造成致突變性上的不同! 再將致突變性最强的鹼性萃取分層經藍棉及高效液相層析等步驟純化後, 測定出其中所含致突變物種類及含量, 其中以 MeIQx 佔最高比例的致突變性(24.0%), 不過以 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) 佔最多量(69.20 ng/g)。

除了中國式食品外, Becher *et al.* (1988) 研究北歐地區的挪威傳統碎肉醬內所含致突變物, 也有一些發現。這種碎肉醬包括 30% 小牛肉、34% 碎豬肉、30% 脫脂奶、4.4% 馬鈴薯澱粉及鹽、香料等, 將其分成兩份, 其中一份另外添加 4.2% 肌酸, 兩份皆置於不鏽鋼盤上以 215°C 煎 20 分鐘後, 再分別經過相同的步驟純化, 來比較兩者產生致突變性的差異, 樣品通過 XAD-2 管柱後以丙酮沖

出，再經半製備高效液相層析的純化，以 TA1538 菌種分別測定，發現未添加肌酸的原始碎肉醬有三個主要致突變性區域，而添加肌酸的樣品也有相似的區域，比較相對三區域的總致突變性，添加肌酸的樣品較未添加肌酸的原始樣品高約 18 倍，由於肌酸的加入並不會生成其他致突變物，所以可使較少量的樣品也能得到多量的致突變物，更重要的是意味著肌酸極有可能是致突變物生成的前驅物！由樣品純化出來的致突變物，以 MeIQ<sub>x</sub> 佔最高致突變性及含量，還有新的致突變物在隨後的研究中確定為 2-amino-3-methylimidazo[4,5-*f*]quinoxaline (IQ<sub>x</sub>) (Fig.1) 及 2-amino-1,6-dimethylimidazo[4,5-*b*]pyridine (1,6-DMIP)，IQ<sub>x</sub> 的致突變性和 MeIQ 相近，而 1,6-DMIP 卻很低（約只為 IQ<sub>x</sub> 的 1/16000），是至目前為止在肉類食物發現致突變物中對沙門氏菌具致突變性最低的（甚至較 PhIP 為低），因此不容易以安氏檢定法測得。

## 二. Maillard 反應參與形成食物致突變物

食物是由許多化學物組成的複雜混合物，例如，胺基酸、醣類、脂質、維生素和礦物質等。這些化合物在一起加熱會產生非常複雜的化學反應，而 Maillard 反應即是其中一種主要的化學反應，能產生一系列的 Maillard 反應產物，此非酵素性褐變反應是 Maillard 在 1912 年所提出，由胺基酸的胺基和醣類的羰基一起加熱生成的化學反應。Maillard 反應在食品加工上，具有相當重大的影響，例如，它會增加食品的風味、口感、質地以及色澤，但

是它亦會造成食品失去原有色澤、味道，同時亦會使蛋白質變性，損耗必須胺基酸等，而使食物的營養價值降低。尤其是 Maillard 反應會產生遺傳毒物，對人類健康造成嚴重傷害 (Nagao *et al.*, 1983; Omura *et al.*, 1983; O'Brien *et al.*, 1988, 1989)。

Hodge (1953) 開始以各種簡單的胺基酸和醣類的模式系統來模仿食品在加熱過程中的化學反應。Spingarn 和 Grivie (1979b) 首先發現 glucose / NH<sub>3</sub> 模式系統的褐變混合物對 TA98 和 TA1538 具有致突變性，他們同時發現其致突變性和 pyrazine 的產量呈線性關係。從此，就有許多學者以各種模式系統和食品在不同條件下進行反應，會產生不同程度的致突變性。例如 Yoshida 和 Okamoto (1980) 將各種胺基酸和葡萄糖的水溶液，煮沸迴流八小時後，十八種胺基酸中，僅有 arginine、lysine 和 cystine 對 TA98+S9 具有致突變性。Jagerstad *et al.* (1983c) 在另一種模式系統測定胺基酸對致突變性的影響，結果顯示僅有 threonine 和 glycine 有致突變性，其餘的幾乎都沒有反應。由以上的實驗，我們可知有關胺基酸在 Maillard 反應的簡單模式系統中，胺基酸對致突變性的影響已有具體的研究。其他尚有許多報告亦在研究不同胺基酸對致突變性的影響 (Powrie *et al.*, 1981; Shinohara *et al.*, 1983; Gazzani *et al.*, 1987)。其他有關水的含量和肌酸的影響也都有深入研究 (Overvik *et al.*, 1989)。

至於 Maillard 反應物在模式系統中對致突變性的影響，至今尚無完整報告，因此值得深入探討。Shibamoto (1983) 由模式系統和肉汁系統中分離確定 300 多種的

Maillard反應物。依其化學構造分爲八大類，有furan、thiophene、thiozole、thiozolidine、pyrazine、imidazole、pyridine和其它異環狀化合物等，其中含量最多且較重要的是pyrazine和imidazole ( Shibamoto,1982 )。在模式系統中至少會生成九十種以上的pyrazine，例如 rhamnose/ $\text{NH}_3$ 模式系統可生成五十一種 pyrazine，佔全部有機萃取物的 80% ( Shibamoto 和 Bernhard,1978 )。Stich *et al.*(1980) 以 Ames test 方法研究發現 pyrazine 和四種 alkyl 衍生物：2-methylpyrazine、2-ethylpyrazine、2,5-dimethylpyrazine 和 2,6-dimethylpyrazine 對 TA100、TA1537 和 TA98 均無致突變性。另外 Shibamoto (1980) 由 cyclotene / $\text{NH}_3$  模式系統中，純化出一種對 TA98 和 TA1538 具有致突變性的 Maillard 反應物—tricyclicpyrazine。在 imidazole 部份，Voogd *et al.*(1979) 曾以 Ames test 方法篩檢各種 imidazole 和 nitroimidazole 的致突變性，發現有 31 種 nitroimidazole 和兩種 imidazole 具有致突變性，而一些 alkylimidazole，例如 1-methylimidazole、1,2-dimethylimidazole、2-methylimidazole、1-methylimidazole、4-methylimidazole 和 4,5-dimethylimidazole 都沒有致突變性。至於其它類的 Maillard 反應物，Shibamoto 在 1989 年的 Review paper 中認爲大多無致突變性。而 Maillard 反應物在食品加工過程中形成致突變物的角色如何？至今亦無報告。但是 Jagerstad 等人 (1983) 首次在 alanine/glucose/creatinine 及 glycine/glucose/creatinine 的模式系統中，探討 Maillard 反應物在形成致突變物上的影響，他們發現分別加入 2-methylpyridine 和 2,5-dimethylpyrazine 可增強 50% 和 80% 的致突變性。

自 1977 年之後，我們已知主要食物致突變物爲 IQ 型

和非IQ型兩類 (Nagao *et al.*,1983; Overvik and Gustafsson ,1990)。Jagerstad *et al.* (1983a)曾提出 Maillard反應物—pyrazine和pyridine參與形成IQ型致突變物的可能化學途徑和其前驅物，他認為pyrazine和pyridine是經由strecker裂解形成amadori化合物，而醛類亦是由胺基酸經strecker裂解生成，至於IQ化合物的imidazole部份，則可能由肌酸轉變成肌酸酐而供給，由以上三類化合物就可合成IQ型致突變物。他所提出的IQ型致突變物形成的可能化學途徑，如Fig.24A所示。接著，在Nyhammar (1986b)的博士論文中則討論到IQ化合物形成的另一可能路徑，指出IQ化合物的imidazole部份可能由肌酸脫水轉變成肌酸酐後先和醛類進行丁醛醇式的凝縮作用 (aldol-type condensation)，然後再和pyrazine或pyridine結合而形成IQ型致突變物。在Jones和Weisburger (1989)的報告也有類似的反應發生，在無醣類的存在下，threonine和acetaldehyde在diethylene glycol-5% H<sub>2</sub>O的狀態下加熱會生成新的致突變物—2-amino-5-ethylidene-1-methylimidazol-4-one(AEMI)及2-amino-1-methyl-5-propylidene-imidazol-4-one(AMPI)，推測其結構是由threonine衍生的醛類和肌酸酐所鍵結形成，此兩致突變物的一些特性如致突變性、具鹼性、抗硝化作用或紫外光吸收光譜等皆和IQ近似，因此作者指出醛類很可能和肌酸酐及另一特定化合物共同以化學反應形成IQ型致突變物。而此一特定化合物很可能是本篇報告所研究的Maillard反應物。

## 貳。中文摘要

豬肉汁經過長時間(12小時)的迴流煮沸(102 °C)後，用二氯甲烷萃取的鹼層物在老鼠肝臟酵素S9的存在下，會對沙門氏菌TA 98產生致突變性，更進一步將豬肉汁的鹼層萃取物以藍棉萃取後，再以半製備型及分析型高效液相層析儀純化收集，並利用菌種特異性及酸性亞硝酸鈉測試加以確認為IQ型致突變物。最後將純化致突變物的紫外線吸收光譜再配合質譜分析確定分別為MeIQx、IQ及MeIQ。估計每克豬肉汁中約含有4.1微克的MeIQx，3.7微克的IQ及12微克的MeIQ，而所含MeIQx、IQ及MeIQ分別相當於全部致突變性的19.0%、27.3%及34.3%，然而豬肉汁中也含有其它致突變物如DiMeIQx(致突變性約佔9.4%)等，但因含量太少而只能以高效液相層析的滯留時間來判別。

至於將豬肉汁中可能產生致突變物的前驅物，如glucose、ribose、alanine、creatinine及Maillard反應物等分別加入豬肉汁模式系統中分析其對豬肉汁形成致突變物的影響，結果顯示glucose、alanine、creatinine及ribose等能增強豬肉汁模式系統之致突變力2.7-6.7倍，Maillard反應物中的2-methylthiophene、3-methylpyridine、2,3-dimethylpyrazine及tetrahydrothiophen-3-one等能促進1.2-2.9倍致突變物之形成，而imidazole和2-acetylpyrrole反而各抑制豬肉汁模式系統之致突變力為0.2和0.3倍，因此我們推測這些會促進豬肉汁模式系統致突變力的化合物很可能參與豬肉汁模式系統形成致突變物的過程。



進一步將對豬肉汁模式系統之致突變力最為增強的兩種 Maillard 反應物—tetrahydrothiophen-3-one 和 2,3-dimethylpyrazine 分別添加入豬肉汁模式系統中探討其最佳反應條件，結果添加 2.5 mmol 的 tetrahydrothiophen-3-one 在 pH 5.0 狀態下煮沸 20 小時能產生最高致突變性，而添加 2.5 mmol 的 2,3-dimethylpyrazine 在 pH 6.0 下煮沸 12 小時則是 2,3-dimethylpyrazine 的最佳反應條件。而在以高效液相層析儀分析所促進形成的主要致突變物亦皆為豬肉汁模式系統中的主要 IQ 型致突變物。同時我們也在添加 tetrahydrothiophen-3-one 或 2,3-dimethylpyrazine 的豬肉汁模式系統中，以氣相層析儀分析其反應過程中 Maillard 反應物的耗用量，發現和致突變物的生成有明顯相關性。因此我們推測此兩類化合物可能是參與豬肉汁模式系統形成致突變物的前驅物。

最後，我們再以 Maillard 反應物／醛類／肌酸酐的簡單的模式系統探討 Jagerstad *et al.* (1983a) 及 Nyhammar (1986) 所提之可能形成 IQ 型致突變物路徑的可能性，結果在 2-methylpyridine／acetylformaldehyde／creatinine 的模式系統中找到每克肌酸酐產生 64 微克的 IQ，因此證實其路徑，不過醛類在此模式系統的角色，仍待進一步的研究。

## 參。緒言

肉類食品在加熱調理過程會產生致突變物或致癌物已被世界各國學者廣泛研究，這些研究大多以牛肉為材料，經過煎、炸和烤等調理方法，產生各種加熱食物致突變物，例如 B[a]P、Trp-P-1、Trp-P-2、Glu-P-1、Glu-P-2、IQ、MeIQ、MeIQx、DiMeIQx 和 PhIP 等 (Kasai *et al.*, 1980, 1981; Felton *et al.*, 1984, 1986, 1988; Becher *et al.*, 1988)。這些化合物大多已被證明為會引起許多器官產生腫瘤的多發性致癌物 (pancarcinogen) (Ohgaki *et al.*, 1984, 1986, 1987; Overvik and Gustafsson, 1990)。因此探討肉類食品加工過程產生致癌物的機轉已為近十年來熱門的研究主題之一。

多年來國外學者對加熱食物所產生致突變物的研究，大多以牛肉為材料。至於有關中國傳統調理—"滷、燉"的方式，是否也會產生致突變性的研究，一直都無報告提出，本研究室 Lee *et al.*(1981)及 Lin *et al.*(1982)首先報告長時間煮沸豬肉汁中有會造成菌種基因結構轉移的前驅致突變物 (frameshift promutagen)，會引起沙門氏桿菌屬 TA98 加上 S9 混合酵素後產生反突變菌落 (revertants)，而影響其形成的因素有肉汁的濃度、酸鹼值及煮沸的時間等，這些促進致突變物形成的影響條件都似乎和加速 Maillard 反應的進行有關，同時亦指出在長時間煮沸豬肉汁中產生的致突變物和當時已知的食物致突變物—Trp-P-1、Glu-P-1 及 B[a]P 等不同，因此本研究擬接續純化及鑑定出長時間煮沸豬肉汁中所含的致突變物。

另外，許多研究結果顯示肉類的一些主要組成份，

例如游離胺基酸、肌酸或肌酸酐和單醣會影響致突變物的形成。由一些簡單的模式系統的研究結果更能明瞭胺基酸的影響，例如 Jagerstad *et al.*(1984) 與 Negishi *et al.*(1985) 在甘胺酸／肌酸酐／葡萄糖的模式系統指出會產生 MeIQ<sub>x</sub> 和 2-amino-3,7,8-trimethylimidazo[4,5-*f*]quinoxaline (7,8-DiMeIQ<sub>x</sub>)，而 Muramatsu 和 Matsushima(1985) 在丙胺酸／肌酸酐／葡萄糖的模式系統發現會產生 MeIQ<sub>x</sub> 和 4,8-DiMeIQ<sub>x</sub>。本研究室先前已發現豬肉汁中，主要形成致突變物的前驅物可能為胺基酸、肌酸酐、單醣和 Maillard 反應物等。在胺基酸方面，除了 cysteine 外，其它 19 種胺基酸和肌酸及各種單醣都能促進致突變物的形成。因此即擬再深入探討主要胺基酸在煮沸豬肉汁模式系統中含量變化對致突變物形成的影響。

再研究 Maillard 反應在此模式系統中和致突變物形成的相關性，分別將六種 Maillard 反應物加入豬肉汁模式系統中，以探討不同類之 Maillard 反應物對此模式系統中致突變物形成之影響，而探討 Maillard 反應物在豬肉汁模式系統的角色。另外，探討不同的反應條件對 Maillard 反應物在豬肉汁模式系統中形成致突變物的影響，以建立最佳反應條件。再以高效液相層析儀分析比較加和不加 Maillard 反應物的豬肉汁模式系統所形成的主要致突變物有何不同？以及 IQ 型和非 IQ 型致突變物之種類與含量。並用氣相層析儀分析豬肉汁模式系統在加熱過程中主要 Maillard 反應物的含量變化，以推測何種 Maillard 反應物會參與致突變物的形成。

總之，本研究擬由進一步純化並確定出長時間煮沸

豬肉汁中所產生的致突變物後，再以各種模式系統探討其形成之因，並探討化學途徑形成IQ型致突變物的可能性。

## 肆。材料與方法

### 一。材料與化學藥品

新鮮里脊豬肉則由台中零售市場購得。果糖、核糖、glucose-6-phosphate (G-6-P)、nicotinamide dinucleotide phosphate (NADP)、copper phthalocyanine cellulose (Blue cotton) 則由美國 Sigma 化學藥品公司購得。二十種胺基酸中，除了脯胺酸、精胺酸等也由美國 Sigma 化學藥品公司購得，其餘胺基酸則由德國 E. Merck 化學藥品公司購得。肌酸酐、葡萄糖、有機溶劑和其它分析級化學藥品也都由德國 E. Merck 化學藥品公司購得。各種 Maillard 反應物則購自美國 Aldrich 化學藥品公司。標準食物致突變物—2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ)、3-amino-1,4-dimethyl-5 *H*-pyrido[4,3-*b*]indole (Trp-P-1)、3-amino-1-methyl-5 *H*-pyrido[4,3-*b*]indole (Trp-P-2) 是由日本東京 Wako 化學藥廠購得。2-amino-3,8-dimethyl-imidazo[4,5-*f*]quinoxaline (MeIQx)、2-amino-3,4-dimethylimidazo[4,5-*f*]quinoxaline (MeIQ)、2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (4,8-DiMeIQx)、2-amino-3,7,8-trimethylimidazo[4,5-*f*]quinoxaline (7,8-DiMeIQx) 是由瑞典 Swedish 大學 Grivas, S. 博士贈與。2-amino-1,6-dimethylimidazo[4,5-*b*]pyridine (DMIP) 及 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) 由美國加州大學 Felton, J.S. 博士贈與。

### 二。豬肉汁模式系統的建立

依照 Lee *et al.*(1981) 及 Lin *et al.*(1982) 的方法製作豬肉汁。在五公斤的新鮮里脊豬肉中加入十公升的蒸餾水煮沸二十分鐘，經玻璃棉過濾後，取水層放入 4°C 冰箱中冷卻後，將凝固的油脂除去再繼續煮沸濃縮至總體積為 1250 ml，即每 25ml 的濃縮豬肉汁中相當於含有 100 g 的豬肉。此 25 ml 的豬肉汁置於迴流裝置中，在 100-105°C 下迴流煮沸 12 小時，此即豬肉汁模式系統。

### 三。致突變性之測定

致突變性之測定是以安氏檢定法測得 (Maron and Ames, 1983)。肝臟酵素上層液 (S9) 是將雄性 Sprague-Dawley 大白鼠腹腔注射 Aroclor 1254 的肝臟製備而得 [以 500 mg/Kg 的劑量將 Aroclor 1254 注射入雄性大白鼠 (180 - 200 g) 腹腔中，直到第四天晚上開始禁食，第五天將老鼠砍頭後在無菌狀態下取出肝臟並依照 Maron and Ames (1983) 的方法與步驟製備 S9 上層液和 S9 混合液]。菌種為需組織胺酸菌種 (histidine auxotroph) — *Salmonella typhimurium* strains TA98 及 TA100 是由美國加州大學柏克萊分校 Ames, B.N. 教授贈與，此菌種之基因標記依照 Maron and Ames (1983) 的方法定期測定其並將其存於液態氮筒中。

### 四。豬肉汁樣品中致突變物的分析

樣品的萃取、純化、分析和鑑定之簡要步驟如Fig. 2所示。

### (一)。豬肉汁樣品的酸鹼分層萃取

將迴流煮沸 12 小時後的濃縮豬肉汁樣品，加入 25 ml 的蒸餾水而稀釋成 2 倍的溶液，以 1 N HCl 調 pH 值為 2.0，再加入  $(\text{NH}_4)_2\text{SO}_4$ ，使溶液飽和以去除蛋白，經玻璃棉過濾蛋白，然後用三倍體積的二氯甲烷和甲醇 (v/v, 2:1) 萃取一次，再以兩倍體積的二氯甲烷萃取兩次，此二氯甲烷層即酸層 (acid fraction)。然後再將水層用 10N NaOH 調 pH 值為 10.0，以三倍體積的二氯甲烷和甲醇 (v/v, 2:1) 萃取一次，再以兩倍體積的二氯甲烷萃取兩次，此時的二氯甲烷層即鹼層 (basic fraction)，酸、鹼層分別加入無水  $\text{Na}_2\text{SO}_4$  吸水再經減壓濃縮乾燥後稱重，並溶於甲醇存於冷凍櫃中，待致突變性的測定和進一步以藍棉 (Blue cotton) 吸附萃取。

### (二)。鹼層以藍棉吸附萃取出粗致突變物

鹼層萃取物經吹乾溶劑後，溶於去離子水成水溶液，依照 Hayatsu *et al.* (1983a,b) 的方法，先加入藍棉在室溫下輕搖 30 分鐘以吸附致突變性多環芳香胺類後，取出藍棉經過清潔去離子水的浸洗，再以濾紙吸乾水分後，利用甲醇和濃氨水 (v/v, 50:1) 溶劑和藍棉輕搖 30 分鐘後，將粗致突變物沖提出來。依上述步驟以新藍棉將原水溶液重複萃取二次，最後將三次沖提液一起減壓濃縮，溶於

1—2 ml 的LC級甲醇中，再以半製備高效液相層析儀純化，其所用的管柱、紫外線波長、移動相和流速如後所述。高效液相層析每分鐘的沖洗液，同樣經過致突變性測定，將具有致突變性的部份收集起來，經濃縮後溶於甲醇中待進一步的實驗。

### (三)。樣品以半製備型高效液相層析儀純化

樣品的半製備高效液相層析儀之純化是用半製備 ODS-FEC 管柱(10  $\mu\text{m}$  particle, 10 x 250 mm, 美國 Regis 公司)分離。此高效液相層析儀是日本 Hitachi D-6500 控制系統，配有 L-4500 型的光二極體陣列光譜偵測器 (photodiode array detector) 及 L-6200 intelligent pump (日本 Hitachi 公司)。沖提液系統為  $\text{CH}_3\text{CN}$  / 10 mM 磷酸-氫氧化鈉 (pH 7.2) 以 gradient 方式沖提，而  $\text{CH}_3\text{CN}$  的比例變化為：0-5 min, 10 to 25% linear gradient, 5-10 min, 25 to 35 % linear gradient, 10-20 min, 35-55% linear gradient, 20-25 min, 55 to 60 % linear gradient, 流速為每分鐘 25 毫升，偵測波長為 200-360nm。收集經過高效液相色層分析儀分離的沖洗液做致突變性測定，將有反應的部份收集起來濃縮後再溶於甲醇中做進一步的分析型高效液相層析儀分析。

### (四)。分析型高效液相層析儀之純化

將經上法純化的樣品注射入 Nucleosil  $\text{C}_{18}$  管柱 (5  $\mu\text{m}$  particle, 4.6 x 250 mm, INPAC Co.) 作進一步純化，其移動相為氘甲烷：水 / 二乙氨 (12: 88 / 0.1, v/v/v)，流速為每分鐘



一毫升。其沖洗液同樣的用自動收集器每分鐘收集起來，分別做致突變性測定及酸性亞硝酸鈉測試，以便和標準食物致突變物的滯留時間及對酸性亞硝酸鈉的反應相互比較，來判定為何類致突變物(IQ型或非IQ型)。

#### (五)。MeIQ<sub>x</sub>、IQ、MeIQ和DiMeIQ<sub>x</sub>等異構物之分析

將上述純化好的樣品注射入 Nucleosil CN管柱 (5 μm particle, 4.6 x 250 mm, INPAC Co.)，移動相為氰甲烷：水／二乙氨／醋酸 [pH6.0] (12 : 88 / 0.1, v/v/v)，流速為每分鐘一毫升 (Becher *et al.*, 1988)。其沖洗液以光二極體陣列光譜偵測器確定主要致突變區分的純度並與 MeIQ<sub>x</sub>、IQ、MeIQ和 DiMeIQ<sub>x</sub> 等標準品比較其紫外線光譜圖，來判定這些主要致突變尖峰是否即為上述之標準品，同時以此區分沖洗液的尖峰面積及對 TA 98 菌種的致突變力來評估其在樣品中之含量。

#### (六)。質譜之測定

EI質譜是以 JEOL Model JMS-DX 300 double-focusing 質譜儀測得。樣品採直接送入 insertion probe，溫度保持在 90°C。70 eV 質譜之 trap current 保持在 300 μA，而 accelerating voltage 為 3 kV。

### 五。豬肉汁之化學成份分析

豬肉汁中之胺基酸、單醣及肌酸酐等化學成份的測定方法分別如下：

(一)。胺基酸的定量(以 Waters 600E 型液相層析儀測得)

分析方法參考 Prieto *et al.* (1990) 所發表的方法

1. 胺基酸標準品以 dansyl chloride derivatization :

20種胺基酸標準品混合液(各0.5 mmol)

↓ 溶於 200 ml 0.01N HCl 中

↓ 取 10 ml 胺基酸標準品混合液

↓ 加 10 ml 1N  $K_2CO_3$

↓ 加 10 ml dansyl chloride 溶液 [0.5 g/25 ml  $(CH_3)_2CO$ ]

↓ 煮沸 2 min

↓ 以 0.45  $\mu m$  濾膜過濾

↓ 以 HPLC 做校正曲線

2. 樣品以 dansyl chloride derivatization :

取長時間煮沸前後的豬肉汁樣品各 2 ml

↓ 加 2 ml 1N  $K_2CO_3$

↓ 加 2 ml dansyl chloride 溶液 [0.5 g/25 ml  $(CH_3)_2CO$ ]

↓ 煮沸 2 min

↓ 以 0.45  $\mu m$  濾膜過濾

↓ 以 HPLC 定量

3. 胺基酸的 HPLC 定量分析條件

Controller : Waters 600E system

Detector : Waters Model 441 ( absorbance at 254 nm )

Column : Merck 50983 LiChrospher 100 RP-18 , 5  $\mu m$  ,

250x4 mm

Mobile phase : 12 mM  $K_2HPO_4$  /  $CH_3COOH$  (pH 6.7) :  $CH_3CN$   
以 gradient 方式沖提，而  $CH_3CN$  的比例變化為 : 0-10 min, 15 to 20% linear gradient , 10-20 min, 20 to 22 % linear gradient , 20-30 min, 22-30 % linear gradient, 30-40 min, 30 to 35 % linear gradient , 40- 50 min, 35 to 45 % linear gradient .

Flow rate : 1.0 ml/min

## (二)。單醣的 HPLC 定量分析條件

分析方法參考 Coppola (1984) 所發表的報告

Controller : Waters 600E system

Detector : Waters Differential Refractometer Electronics Unit & R  
401 ( Refractive Index )

Column : Interaction Chromatography Inc. CHO-620 carbohydrate  
column (cat.no.25210) , 300x6.5 mm

Mobile phase : deionized water

Flow rate : 0.6 ml/min

Temperature : 90°C

Sensitivity : 16x

## (三)。肌酸酐(creatinine) 動力學定量

原理為 Jaffe kinetic reaction, 分析方法參考 Larsen (1972) 的方法

Absorbance wavelength : 510 nm

Temperature : 30°C

Measure against water

試劑製備：將 Alkaline reagent 和 picric acid 等量混合為 working reagent

以水歸零

取 1 ml working reagent 至 cuvette 中 (30°C)

加入 0.05 ml 的標準品或樣品 (1000 倍稀釋)

混合均勻 30 秒後，讀取 510 nm 吸光度為 Absorbance I

再經 30 秒讀取 510 nm 吸光度為 Absorbance II

$\text{Absorbance II} - \text{Absorbance I} = \Delta A$

計算濃度：

Unknown concentration =

$\Delta A \text{ of unknown} / \Delta A \text{ of standard} \times \text{standard concentration}$

## 六。豬肉汁模式系統中 Maillard 反應物的分析

### (一)。Maillard 反應物的萃取

萃取方法參考 Shibamoto (1982) 所提的萃取方法

各種反應條件所得之樣品分別以 100 ml 二氯甲烷萃取，然後離心而分層，再重複萃取三次，取四次所得二氯甲烷層予以減壓濃縮乾燥，溶於 2 ml 甲醇後通過 0.2  $\mu\text{m}$  濾膜，最後將樣品體積濃縮至 50  $\mu\text{l}$ ，再以氣相層析儀分析。

## (二)。以氣相層析儀分析 Maillard 反應物

分析方法參考 Hong and Richard (1992) 所發表文獻

氣相層析儀：Varian STAR 3400

Polar column：Chrompack WCOT fused silica coating CP-WAX

52CB capillary column (50 m x 0.32 mm i.d.)

Detector：flame ionization detector (F.I.D.)

Carrier gas：N<sub>2</sub>, 0.8 ml/min.

Injector and Detector temp.：250°C

Temp. program：70°C (8 min)→2°C/min→160°C (60min)

## (三)。Maillard 反應物之反應條件對豬肉汁模式系統形成致突變物的影響

在 25 ml (相當於 100 g 豬肉) 的豬肉汁模式系統中，加入六類 Maillard 反應物 (各 5 mmol)，迴流煮沸 12 小時後，測定萃取鹼層的致突變性，其致突變物之萃取方法參見四之一。

為探討產生最佳致突變性的反應條件，擬分別研究反應的 pH 值、反應時間和反應物濃度的影響。

### 1. pH 值的影響：

將加有 Maillard 反應物的豬肉汁模式系統以 NaOH 或 HCl 調反應混合液之 pH 值分別為 3、5、7、9、11 等，在 100-

105 ℃下迴流加熱12小時後，萃取每一反應 pH 值樣品做致突變性檢測並取少量樣品做褐變程度的測定。

## 2. 反應時間的影響：

將加有 Maillard 反應物的豬肉汁模式系統在 100-105 ℃ 狀態下加熱 1、2、4、8、12、16、20 小時，萃取每一反應時間樣品做致突變性檢測並取少量樣品做褐變程度的測定。

## 3. 反應物濃度的影響：

分別取 2.5、5、10、20 mmol 的 Maillard 反應物加到 25 ml 的豬肉汁模式系統，迴流加熱 12 小時，萃取每一反應濃度樣品做致突變性檢測並取少量樣品做褐變程度的測定。

## 七. 褐變之測定

將煮沸 12 小時加有各種可能前驅物的豬肉汁樣品，用蒸餾水稀釋十倍後，以紫外線光譜儀 (Shimadzu UV-2100) 於 420 nm 波長測定吸光度。

## 八. 簡單模式系統樣品中的致突變物分析

### (一)。簡單模式系統樣品的酸鹼分層萃取

添加 Maillard 反應物、醛類及肌酸酐於二乙醯甘醇—水溶液 (DEG-5% $H_2O$ ) 迴流 1 小時後的混合樣品 (105 ml)，加入 420ml 的蒸餾水而稀釋成 5 倍的溶液，然後通過 XAD-2 column，分別以 200 ml 丙酮、甲醇沖提之，所得之溶劑經減壓濃縮乾燥後溶於 50ml 的去離子水中，其後之酸鹼分層萃取參見四之(一)。

### (二)。簡單模式系統樣品之純化

鹼層以藍棉吸附萃取的步驟同前述四之(二)的方法，由藍棉萃取所得之濃縮物再利用薄層色層分析法 (Thin-layer Chromatography, TLC)，將樣品作進一步純化。薄層色層分析法是將藍棉萃取後之濃縮物和 IQ、MeIQ<sub>x</sub>、MeIQ 及 DiMeIQ<sub>x</sub> 等標準品分別點於製備型 E Merck Kieselgel 60 F<sub>254</sub> 薄層分析片上，再利用甲醇和氯仿 (methanol-chloroform)(10:90, v/v) 將樣品依其極性不同而拉開，然後分別在 254 及 366 nm 等波長下觀察其層析圖，以在 366 nm 吸收所呈現的圖形分為 B 個區域 (band)，刮下各區域分別以甲醇和氯仿 (10:90, 50:50, 100:0, v/v) 將樣品洗出後，各取量測定致突變性，再將有反突變數反應的區域混合濃縮，接著以半製備型高效液相層析儀再進行純化。

### (三)。樣品以半製備型高效液相層析儀純化

經薄層色層分析法純化後的樣品再以半製備型高效液相層析儀純化 (同前述四之(三)的方法)，收集與 IQ

標準品相同滯留時間的區域，濃縮之後再以分析型高效液相層析儀配合 Nucleosil CN 管柱之分析（同前述四之(四)的方法）。

#### (四)。樣品中 IQ 之分析鑑定

經過二次 Nucleosil CN 管柱純化的樣品再以 Spherisorb pH Stable C<sub>18</sub> 管柱 (5 μm particle, 4.6 x 250 mm, INPAC Co.)，沖提液為氟甲烷：水／二乙氨 (12: 88/ 0.1, v/v/v)，流速為每分鐘一毫升。其沖洗液以光二極體陣列光譜偵測器確定主要致突變區分的純度並與 IQ 標準品比較其紫外線光譜圖，來判定這些主要致突變尖峰是否即是 IQ，同時以此區分沖洗液的尖峰面積及對 TA 98 菌種的致突變力來評估其在樣品中之含量。

#### (五)。質譜之測定

質譜之測定方法與四之(六)相同。



## 伍。結果與討論

### 一。豬肉汁萃取物中主要致突變物之鑑定：

長時間煮沸豬肉汁萃取後的酸層經安氏檢定法測定，發現並不具致突變性，而其鹼層粗萃取物經過藍棉的吸附再萃取後，將由甲醇和濃氨水沖提所得之濃縮物再以高效液相層析之半製備型管柱大量分離純化樣品，並測定其沖提液之致突變性，其層析圖及以安氏檢測法測得之反突變數區分 (Revertants fraction) 圖見 Fig. 3，顯示具有較高致突變性的區域主要落在 MeIQ<sub>x</sub>、IQ 和 MeIQ 等標準品的滯留時間 (Retention time) 附近，再將具有較高致突變性的區域收集濃縮，進一步經分析型 C<sub>18</sub> 管柱以高效液相層析儀分析結果如 Fig. 4 所示，其第 16 分鐘左右的致突變性區域與 MeIQ<sub>x</sub>、IQ 的滯留時間相近，而第 31 分鐘的區域則和 MeIQ 相近，另外在第 26 分鐘的區域其致突變性較弱，由其滯留時間推測為 DiMeIQ<sub>x</sub>，接著將前述主要致突變性區域分別做菌株致突變特異性及酸性亞硝酸化反應之耐受性測試，結果列於 Table 1，顯示由樣品所得之致突變區分和 IQ 型致突變物標準品的反應特性一致，而與非 IQ 型致突變物 - Tip-P-2 的反應特性差異較大，特別是 IQ 型致突變物在酸性下不會被亞硝酸鈉作用而減低致突變力但非 IQ 型致突變物則會，因此我們所純化的致突變物都是屬於 IQ 型致突變物。

然後又將前述主要致突變性部分予以濃縮後，再以分析型 CN 管柱配合另一種移動相，將 C<sub>18</sub> 管柱所無法完

全分開的致突變物—MeIQ<sub>x</sub>和IQ完全分開，使得在層析圖上可看出三個主要波峰，藉由其滯留時間 (Fig. 5) 分別與標準品相互對照，及以光二極體陣列偵測出紫外線吸收光譜 (Fig. 6) 其最大吸收波長分別為 272.5 nm、260.8 nm 及 262.6 nm 等都和標準品完全一致，最後再以質譜 (Mass spectrum) 確定其質量，前三種純化致突變物都與標準品之主要尖峰相符，*m/z* 分別為 213、198 及 212 (Fig. 7, 8, 9)，由上述結果可知長時間煮沸豬肉汁中主要致突變物為 MeIQ<sub>x</sub>、IQ 及 MeIQ，還有以安氏檢定法測到在 MeIQ<sub>x</sub> 和 IQ 之間的致突變性區域可能為 DiMeIQ<sub>x</sub> (Fig. 5)，以上各種致突變物所佔致突變力及含量列於 Table 2。表中分析煮沸豬肉汁中會生成 MeIQ<sub>x</sub>、IQ、MeIQ 和 DiMeIQ<sub>x</sub> 等四種 IQ 型致突變物，佔所有致突變力的 90.0%，其中以 MeIQ<sub>x</sub>、IQ、MeIQ 等三種共佔 80.6%，分別為 19.0%、27.3% 和 34.3%。再以高效液相層析儀定量曲線測定 MeIQ<sub>x</sub>、IQ、MeIQ 等三種致突變物的含量，每克肉中分別含有 4.1、3.7 及 1.2 微克。

由 Table 3 比較各種肉類食物中所含的 IQ 型致突變物，發現我們所測定豬肉汁中的 MeIQ<sub>x</sub> 含量較煎碎牛肉 (Hargraves and Pariza, 1983; Felton *et al.*, 1984; Murray *et al.*, 1988)、煙燻鯖魚乾 (Kato *et al.*, 1986) 為高，但比食用的牛肉萃取物來的低 (Hargraves and Pariza, 1983)；而 IQ 的含量也高於煎碎牛肉 (Barnes *et al.*, 1983; Felton *et al.*, 1984)、烤牛肉和鮭魚 (Yamaizumi *et al.*, 1986)，但比烤沙丁魚 (Kasai *et al.*, 1981; Yamaizumi *et al.*, 1986) 為低；至於 MeIQ 的量則較煎碎牛肉 (Felton *et al.*, 1984) 為高，而比烤沙丁魚、烤鮭魚低

(Yamaizumi *et al.*, 1986)。

由此可見以長時間煮沸的加熱方式所產生的致突變物含量並不會比短時間煎、烤方式低，且中國人向來特別喜愛滷、燉的食物，此與國人在消化道癌症的發生率之相關性，實在值得再做深入之探討！

另外，在 Table 4 將各種加熱食物所含的致突變物或致癌物作一整理，其中以豬肉為研究材料的報告很少，且並未定出豬肉中致突變物的含量，僅提到煎豬肉會產生 IQ、MeIQ、MeIQx、DiMeIQx 及 PhIP 等已知致突變物 (Vahl *et al.*, 1988)，其結果和我們的非常近似，只是油煎的方式和水煮的方式無論在溫度、加熱時間及水含量等都有些差別，但仍可看出煎豬肉或長時間煮沸豬肉汁，都會生成 IQ、MeIQ、MeIQx 及 DiMeIQx 等已知 IQ 型致突變物。

## 二。豬肉汁之游離胺基酸、單醣和肌酸酐在煮沸前後的變化及其對致突變物形成之影響：

至於長時間煮沸豬肉汁為何會產生致突變物之研究？首先探討其化學組成份—游離胺基酸是否和致突變物形成有某種程度的關係，因此分析豬肉汁中游離胺基酸在煮沸前後的含量變化，結果如 Fig.10 和 Table 5 顯示由豬肉汁本身的胺基酸組成來看，其含量變化佔胺基酸總量改變 1% 以上的胺基酸依次為 His、Ala、Pro、Met、Asn、Tyr、Arg、Ser、Val、Gly 和 Lys 等。於是添以可能參與反應之前驅物的方式，將變化較大的十二

種胺基酸分別添加 2 mmol 在豬肉汁中煮沸，結果以安氏檢定法分別檢測添加 Ala、Tyr、Gly、Asn、Leu、Pro 等胺基酸的混合液，其致突變性皆促進三倍以上 (Table 6)，再將添加 Ala 的豬肉汁萃取鹼層以高效液相層析儀分析，發現在相同區域的致突變性皆較豬肉汁本身促進 1.3-5.2 倍 (Fig.11; Table 7)，而 Ala 在豬肉汁煮沸前後變化量又佔第二位，可見 Ala 是煮沸豬肉汁中形成致突變物的重要前驅胺基酸。

至於單醣和肌酸酐在豬肉汁煮沸前後的含量如 Fig.12 和 Table 8 所示，表中就單醣中的葡萄糖、果糖及核糖而言，以葡萄糖的變化較大，核糖次之，然後為果糖，另外以肌酸酐的減少量來看也有近 60%，此亦說明的葡萄糖、果糖、核糖及肌酸酐等都可能參與形成致突變物之 Maillard 反應。

Table 9 中將各類可能為豬肉汁形成致突變物的前驅物分別添加於豬肉汁中煮沸，以檢測其致突變力的增減：在添加 2 mmol 的葡萄糖及核糖會促進 3 倍及 7 倍的致突變性，有趣的是添加核糖的致突變性竟較添加葡萄糖強約 2.5 倍；而添加 17.5 mmol 的肌酸酐也促進致突變性 5 倍。再將核糖、丙胺酸、肌酸酐及 Maillard 反應物—tetrahydrothiophen-3-one 同時添加在水和豬肉汁中煮沸也都促進其致突變性 11 倍和 22 倍，因此表示這些化合物都可能是致突變物形成的重要前驅物。

就豬肉汁煮沸前後胺基酸變化量的測定而言，以高效液相層析儀分析得 Ala、Pro、Asn、Tyr、Ser、Thr、Leu 和 Gly 等變化較大，而 Macy *et al.* (1964) 的報告中利用

離子交換層析法測定豬肉粉末加熱前後胺基酸的變化量，其中上述胺基酸的變化量也較大，此與我們的結果相當接近。另外，在單醣的變化上，豬肉汁煮沸前後以葡萄糖的變化較大，核糖次之，然後為果糖，而 Macy *et al.* (1964) 測定單醣的變化量大小依次亦為葡萄糖、核糖和果糖，又與我們的測定相吻合，可見我們對胺基酸及單醣的定量方法應屬準確。

有關胺基酸影響致突變物形成的影響，Yoshida and Okamoto (1980) 將各種胺基酸和葡萄糖溶於水中煮沸八小時後，以二氯甲烷萃取其鹼層做致突變性測定，結果發現只有 Arg 和 Lys 對 TA98 加上 S9 會產生致突變性。Omura *et al.* (1983) 也以同樣的模式系統做致突變物形成的實驗，結果顯示除了 Arg、Lys 之外，還增加了 Gly 和 Ile 具有致突變性。Jagerstad *et al.* (1983) 以另一種模式系統研究胺基酸對形成致突變物的影響，他們將胺基酸、肌酸和葡萄糖溶於含 14% 水的二乙醯甘醇溶液 (DEG-14% $H_2O$ ) 中迴流兩小時後，直接以反應混合液做致突變性測定，則僅有 Thr 和 Gly 具有較強的致突變性，由上述實驗可知肌酸的加入與否可能會影響胺基酸在簡單模式系統產生致突變性。

由 Table 10 可看出 alanine 在簡單模式系統中證明會產生 MeIQx、MeIQ 及 4,8-DiMeIQx 等致突變物 (Grivas *et al.*, 1985; Muramatsu *et al.*, 1985; Grivas *et al.*, 1986; Overvik *et al.*, 1989)，proline 會產生 IQ (Yoshida *et al.*, 1984)，而 tyrosine 則產生 MeIQx (Overvik *et al.*, 1989)，serine 會生成 MeIQx 及 IQ (Overvik *et al.*, 1989; Knize *et al.*, 1988a)，還有 glycine 也會在模式系統中生成

MeIQx、IQ及7,8-DiMeIQx (Jagerstad *et al.*,1984; Grivas *et al.*,1986; Negishi *et al.*,1984 )，這些胺基酸正是豬肉汁中變化較大的，因此認為這些胺基酸可能是豬肉汁煮沸時形成致突變物的重要前驅物。

以上實驗都是在簡單的模式系統中得到的結果，若在複雜的肉汁系統中，胺基酸對致突變物形成的影響如何？Overvik *et al.* (1989) 分別以十五種胺基酸和肌酸混合在碎豬肉中乾煎的模式系統來探討胺基酸對致突變物形成的影響。其結果亦指出外加胺基酸的種類對碎豬肉產生致突變物的種類和含量有密切的關係，結果顯示 Thr 會增強其致突變性43倍，其中有三種胺基酸—Ser、Ala和 Tyr 會生成MeIQx，而在豬肉汁模式系統中，我們發現加入 alanine 也會促進IQ型致突變物的生成。不過，由 Taylor *et al.* (1986) 的牛肉汁模式系統之實驗結果發現和我們的煮沸豬肉汁模式系統仍有些差異，因為他們發現外加胺基酸於牛肉汁中，僅有 Trp、Cys 和 Pro 會增強牛肉汁兩倍以上的致突變性，而 Ala、Tyr 及 Gly 並無此反應，可見牛肉汁與豬肉汁模式系統就胺基酸來看還是不盡相同，以致其形成的致突變物會有些差異。

綜合以上的研究結果，我們可歸納出有關胺基酸及單醣等致突變物前驅物的結論為：豬肉汁在煮沸前後以 Ala、Pro、Tyr、Ser 和 Gly 等變化較大且都會形成IQ型致突變物；而單醣中則以葡萄糖、核糖、果糖等變化較大。因此這些化合物都可能成為豬肉汁形成IQ型致突變物的重要前驅物。

### 三。豬肉汁中 Maillard 反應物在煮沸前後的變化和其添加於豬肉汁形成致突變物的最佳反應條件之研究：

28種 Maillard 反應物標準品之氣相層析圖如 Fig.13 所示，而豬肉汁煮沸過程中所生成之 Maillard 反應物的消長變化與致突變物形成之相關性，以氣相層析儀測到豬肉汁模式系統本身在未開始長時間煮沸時已生成 2,3-dimethylpyrazine 和 tetrahydrothiophen-3-one (Fig.14B)，而隨著加熱時間的增加可看出兩種 Maillard 反應物含量的變化 (Fig.14 C, D, E, F, G, H, I)。分別在煮沸0、1、2、4、8、12、16及20小時予以測定，顯示 2,3-dimethylpyrazine 在各時間的含量變化與其致突變性的高低無負相關性 ( $r = -0.31, P > 0.05$ )，而 tetrahydrothiophen-3-one 的含量變化與其致突變性的高低則呈些微負相關性 ( $r = -0.82, P < 0.1$ )，若將 2,3-dimethylpyrazine 和 tetrahydrothiophen-3-one 的變化量一起計算時，則和致突變性也呈些微負相關性 ( $r = -0.71, P < 0.1$ ) (Table 11)，表示 2,3-dimethylpyrazine 和 tetrahydrothiophen-3-one 的變化量和煮沸豬肉汁致突變性的形成有相關性。再分析添加有 2,3-dimethylpyrazine 或 tetrahydrothiophen-3-one 的豬肉汁模式系統中，此兩種 Maillard 反應物的消長變化與致突變物形成之相關性，Table 12 結果顯示添加 5mmol 的 2,3-dimethylpyrazine 之豬肉汁模式系統中，該 Maillard 反應物的含量會隨著煮沸時間的延長而呈遞減，而和致突變性的增高有顯著負相關性 ( $r = -0.85, P < 0.01$ )，即當致突變性增高時 2,3-dimethylpyrazine 的含量就會減少；而添加 5 mmol 的 tetrahydro-

thiophen-3-one 其含量也會隨著煮沸時間的延長亦呈遞減，也有顯著負相關性 ( $r = -0.83, P < 0.02$ ) (Table 13)，由此更可推測 2,3-dimethylpyrazine 和 tetrahydrothiophen-3-one 確實參與煮沸豬肉汁模式系統中形成致突變物。

再將豬肉汁中測到有含量變化的 2,3-dimethylpyrazine 以及 tetrahydrothiophen-3-one，再選擇其他類 Maillard 反應物如 3-methylpyridine、2-methylthiophene、2-acetylpyrrole 及 imidazole 等 (Fig. 15)，分別加入豬肉汁中煮沸 12 小時後，測定其鹼層萃取液的致突變性，由 Table 14 的結果顯示 Maillard 反應物中的 thiophene、pyrazine 和 pyridine 類較其他類化合物較能促進致突變物的形成，其中以 thiophene 類化合物之 tetrahydro-thiophen-3-one 最能增強豬肉汁模式系統之致突變力 2.90 倍，其次分別為 2,3-dimethylpyrazine、3-methylpyridine、2-methylthiophene 等，能增強 1.64-1.23 倍，因此我們推測這些化合物可能會參與豬肉汁模式系統形成致突變物。而 2-acetylpyrrole 和 imidazole 反而抑制致突變性為 0.34 和 0.20 倍，則推測此兩種 Maillard 反應物可能產生對致突變物形成的抑制化合物或改變致突變物形成的化學反應路徑，至於其確實抑制機轉則有待進一步的研究。

為了探討 Maillard 反應物是否確實參與豬肉汁模式系統形成致突變物，我們進一步以高效液相層析儀純化分析豬肉汁和添加不同 Maillard 反應物之萃取物中 IQ 型致突變物的含量 (Fig. 16, 17)，結果如 Table 15 所示，顯示添加不同 Maillard 反應物之高效液相層析圖和豬肉汁的鹼層萃取物之層析圖大體上相似。但是其每一致突變區分的致突變性強弱變化，則隨 Maillard 反應物種類不同而異。添



加 tetrahydrothiophen-3-one 所生成的 IQ 型致突變物含量增加最多，其次為 2,3-dimethylpyrazine，而 2-acetylpyrrole 和 imidazole 都不會增加 IQ 型致突變物的含量，反而會減低其含量，因此我們可推測 tetrahydrothiophen-3-one 和 2,3-dimethylpyrazine 可能是煮沸豬肉汁模式系統形成致突變物的前驅物。

由上述的結果可知 tetrahydrothiophen-3-one 和 2,3-dimethylpyrazine 較能增強豬肉汁模式系統之致突變力，因此擬採用上述兩種 Maillard 反應物進一步探討不同 Maillard 反應物在豬肉汁模式系統的酸鹼值、Maillard 反應物濃度及煮沸時間等反應條件下的影響，並找出對豬肉汁形成致突變物的最佳反應條件，以便純化分析其中之微量致突變物。由 Table 16 可知添加 2,3-dimethylpyrazine 和 tetrahydrothiophen-3-one 到豬肉汁模式系統中，在 pH 3.0, 5.0, 6.0, 7.0, 9.0 和 11.0 的狀態下，以添加 tetrahydrothiophen-3-one 在 pH 5.0 和 9.0 時最好，而添加 2,3-dimethylpyrazine 則以 pH 6.0 和 9.0 時最能增強其致突變性，此現象和沒有添加 Maillard 反應物的豬肉汁模式系統之最佳 pH 值相似，因此可推測添加 2,3-dimethylpyrazine 或 tetrahydrothiophen-3-one 到豬肉汁模式系統中並不會改變其形成致突變物之化學反應條件。可是在 pH 9.0 時褐變強度 (browning intensity) 值卻偏低，此結果或許可解釋為褐變反應在各種 pH 值的豬肉汁模式系統中並非形成致突變物之主要化學反應途徑，此乃可能由於調整 pH 值時引起豬肉汁中蛋白質或其他含氮物的變性所致。

Table 17 顯示添加 2,3-dimethylpyrazine 和 tetrahydro-

thiophen-3-one 到豬肉汁模式系統中產生最強致突變性的煮沸時間並不相同，後者和沒有添加 Maillard 反應物的豬肉汁模式系統產生最強致突變性的煮沸時間一致都是 20 小時，而前者則僅須 12 小時即達到最高的致突變性。由添加 tetrahydrothiophen-3-one 的實驗可知僅需煮沸 1 小時即開始生成致突變物，而豬肉汁本身和添加 2,3-dimethylpyrazine 的模式系統都必須煮沸 8 小時才產生致突變物。有趣的是添加 2,3-dimethylpyrazine 煮沸時間延長到 20 小時，其致突變性非但沒有增加反而減低，這顯示 2,3-dimethylpyrazine 煮沸到後期則會抑制豬肉汁模式系統形成致突變物。而研究其致突變性和褐變強度間的關係上，發現對豬肉汁本身呈現明顯之相關性 ( $r = 0.82, P < 0.05$ )，而對豬肉汁添加 2,3-dimethylpyrazine 的模式系統也有明顯之相關性 ( $r = 0.83, P < 0.05$ )，至於對豬肉汁添加 tetrahydrothiophen-3-one 的模式系統更有顯著之相關性 ( $r = 0.85, P < 0.02$ )，可見褐變反應的確參與以上模式系統的致突變物形成。

至於添加到豬肉汁模式系統中的 Maillard 反應物濃度對致突變物形成之影響如 Table 18 所示，無論 2,3-dimethylpyrazine 或 tetrahydrothiophene-3-one 都以添加 2.5 mmol 時產生最強的致突變性。而添加不同濃度 tetrahydrothiophen-3-one 對豬肉汁模式系統致突變物之形成比添加 2,3-dimethylpyrazine 具有較明顯的影響。再研究其致突變性和褐變強度間的關係上，發現對豬肉汁添加各種濃度 2,3-dimethylpyrazine 的模式系統並無相關性 ( $r = 0.21, P > 0.05$ )，至於對豬肉汁添加 tetrahydrothiophen-3-one 的模式系統則有明顯相關

性 ( $r = 0.91, P < 0.05$ ), 可見在反應的濃度條件上, 褐變反應只參與添加 tetrahydrothiophen-3-one 的模式系統之致突變物形成。雖然至今尚無其他文獻報告實驗證明 Maillard 反應物可做為形成 IQ 型致突變物的前驅物, 不過由我們的各種結果顯示 tetrahydrothiophen-3-one 或許是形成 IQ 型致突變物之前驅物。

而關於兩種 Maillard 反應物添加量的消長變化與致突變物的形成之比較, 雖然由 Table 11 看不出其變化量與致突變物形成的相關性, 但在 Table 13 及 Table 14 顯示添加較大量 Maillard 反應物時, 其變化量與致突變物形成則有顯著相關性, 所以 Table 11 的結果是由於豬肉汁本身的 Maillard 反應物含量不高所以導致變化不明顯。另外在 Table 13 及 Table 14 還可看出在相同添加量下, tetrahydrothiophen-3-one 的消耗量明顯要比 2,3-dimethylpyrazine 為大, 此亦呼應前面所提到 tetrahydrothiophen-3-one 的濃度對豬肉汁模式系統致突變物之形成比添加 2,3-dimethylpyrazine 具有較明顯影響的結果。

至於將豬肉汁及豬肉汁分別添加 2,3-dimethylpyrazine 和 tetrahydrothiophen-3-one 等三種模式系統調成六種 pH 值來煮沸時, 豬肉汁本身的致突變性以在 pH 9.0 最高, 此與 Lin *et al.* (1982) 及 Taylor *et al.* (1986) 的報告都近似。而添加 2,3-dimethylpyrazine 和 tetrahydrothiophen-3-one 的模式系統, 在 pH 9.0 時的致突變性也有較高反應性, 乃由於傾鹼性狀態會促進 Maillard 反應之進行, 因此將 2,3-dimethylpyrazine 和 tetrahydrothiophen-3-one 在 pH 9.0 時添加入豬肉汁中煮沸會促進其致突變物的生成, 即表示 Maillard 反應物可能參與豬

肉汁產生致突變物的部份化學路徑。

Table 16 顯示豬肉汁在較佳反應 pH 值 (pH 5.0) 分別添加 2,3-dimethylpyrazine 及 tetrahydrothiophen-3-one 時會造成最低及最高致突變性，而 Taylor *et al.* (1986) 也在牛肉汁次佳反應 pH 值 (pH 4.0) 分別添加 pyrazine、2-methylpyrazine 及 2,5-dimethylpyrazine 等三種 Maillard 反應物，結果都無致突變性產生，此與我們在豬肉汁中添加 2,3-dimethylpyrazine 的結果近似，但與添加 tetrahydrothiophen-3-one 的反應則差異極大，顯示不同種類的 Maillard 反應物在相同 pH 值下會促進或抑制致突變性的產生。至於豬肉汁和牛肉汁的致突變性與煮沸時間曲線比較，由 Table 17a 和 Lin *et al.* (1982) 及 Taylor *et al.* (1986) 的報告都顯示一致的趨勢。

總括上述實驗的結果，tetrahydrothiophen-3-one 和 2,3-dimethylpyrazine 是豬肉汁煮沸過程中偵測到變化最明顯的兩種 Maillard 反應物，以添加的方式研究在酸鹼值、Maillard 反應物濃度及煮沸時間等反應條件下的影響，其反應趨勢都和豬肉汁的反應近似，尤其以 tetrahydrothiophen-3-one 最為接近，即當豬肉汁中添加 25 mmol 的 tetrahydrothiophen-3-one 在 pH 5.0 狀態下煮沸 20 小時能產生最高致突變性，同時亦會生成最大量的 IQ 型致突變物。而添加 2.5 mmol 的 2,3-dimethylpyrazine 在 pH 6.0 下煮沸 12 小時則是 2,3-dimethylpyrazine 的最佳反應條件。

#### 四。2-Methylpyridine / acetylformaldehyde / creatinine 模式系統形成 IQ 致突變物之研究

將 2-methylpyridine / acetylformaldehyde / creatinine 模式系統之反應混合液通過 XAD-2 管柱，所得之沖提液經酸鹼分層萃取得其鹼層，再經過藍棉的吸附後，將所得之濃縮物以 TLC 展開如 Fig. 18，以甲醇 / 氯仿之混合液洗下有致突變性反應的區域，再以高效液相層析之半製備型管柱大量分離純化粗樣品，並測定其沖提液之致突變性，其層析圖及以安氏檢測法測得之反突變數區域圖見 Fig. 19，顯示在 IQ 和 MeIQ 等標準品的滯留時間附近有致突變性的區域，另外在其後的滯留時間還有更高的致突變性區域沒有標準品可以對照而為未知化合物，由於本實驗目的主要為證明 IQ 型致突變物的形成路徑，因而將可能為 IQ 型致突變物的滯留區域收集濃縮，進一步經分析型 CN 管柱以高效液相層析儀分析結果如 Fig. 20 所示，其第 21 分鐘左右的致突變性區域和 IQ 的滯留時間相近，另外在第 33 分鐘的區域其致突變性較弱，可能為 MeIQ，再將前述主要致突變性區域予以濃縮後，再以分析型 CN 管柱純化一次，使得在樣品的層析圖上可看到 IQ 的波峰 (Fig. 21)，再把此波峰區域收集濃縮後以 pH stable C<sub>18</sub> 管柱作最後分析，其滯留時間 (Fig. 22) 分別與 IQ 標準品相互一致，及以光二極體陣列偵測出紫外線吸收光譜 (Fig. 23)，樣品和 IQ 標準品的最大吸收波長分別為 261.7 nm 及 261.3 nm，最後以質譜確定純化致突變物的 m/z 為 198，其他裂解物之 m/z 也都與標準品之 m/z 相符 (Fig. 24)，而分析出此模式系統會生成 IQ，經計算在每毫莫耳肌酸酐所產生的 IQ 量約為 6.4 微克。

Jagerstad *et al.* (1983) 最先添加 2-methylpyridine 和 2,5-di-

methylpyridine 到 glycine/glucose/ creatinine 及 alanine/glucose /creatinine 模式系統中分別會增強其致突變力約 50% 及 80%，因而提出 pyridine 類化合物形成 imidazolquinoline (IQ) 類致突變物的可能化學途徑，路徑中異環胺的 imidazole 部分可能由 creatinine 構成，而其他兩芳香環部分則可能由 Maillard 反應物經由 Strecker 裂解形成 Amadori 化合物後，再與由氨基酸裂解所得的醛類結合形成 (Jagerstad *et al.*, 1983, Nyhammar, 1986)。至此，我們已經證明在 2-methylpyridine / acetylformaldehyde / creatinine 模式系統會形成 IQ 型致突變物，也就是證明 Jagerstad *et al.* (1983a) 及 Nyhammar (1986) 所提形成 IQ 型致突變物的路徑 (參見 Fig. 25 A,B) 和可能性，不過以本實驗的結果顯示以路徑 B 的可能性較大，因為在初步純化時 (Fig. 18, 19) 所呈現較大致突變性的部分，推測可能是 Jones *et al.* (1989) 所發現的致突變物— 2-amino-5-ethylidene-1-methyl-imidazol-4-one (AEMI) 或 2-amino-1-methyl-5-propylideneimidazol-4-one (AMPI)，其在無醣類的存在下，由 threonine 和 acetaldehyde 在 diethylene glycol-5% H<sub>2</sub>O 的狀態下加熱生成，因此推測其結構是由 threonine 衍生的醛類和肌酸酐所結合形成，此兩致突變物的一些特性如致突變性、具鹼性、抗硝化作用或紫外光吸收光譜等皆和 IQ 近似，因此作者指出醛類很可能和肌酸酐及另一特定化合物共同以化學反應形成 IQ 型致突變物。而此一特定化合物很可能是本篇報告所研究的 Maillard 反應物。於是我們推測只當 Maillard 反應物和 AEMI 或 AMPI 相結合後才會生成 IQ 型致突變物。

另外，我們又試著採用其他醛類化合物加入反應以

比較其影響，在 Table 19 的結果中顯示致突變力的形成與醛類的種類有著極大的關係，再參考 Fig. 26 的醛類結構圖可看出醛類的碳數越多，其所產生的致突變性也越高，只是不同 Maillard 反應物的影響，則有待更進一步的研究。至於在沒有醛類存在時，例如 tetrahydrothiophen-3-one 和 creatinine 反應也會形成致突變物，經過藍棉的萃取以及高效液相層析之半製備型管柱分離純化後，顯示未加醛類的反應就無法形成 IQ 型致突變物 (Fig. 27)，而兩種反應形成致突變物的確實結構以及醛類在此模式系統的角色，仍待進一步的研究。另外，由於上述模式系統所形成的 IQ 量較低且純化步驟繁複困難，在後續的實驗將採用放射性標定化合物以利研究之進行。

## 陸。 參考文獻

- Ames, B.N., Lee, F.D. and Durston, W.E. (1973) An improved bacterial test system for the detection and classification of mutagens and carcinogens. *Proc. Natl. Acad. Sci. U.S.*, 70, 782-786.
- Ames, B.N., McCann, J. and Yamasaki, E. (1975) Methods for detecting carcinogens and mutagens with the *Salmonella* / mammalian microsomes mutagenicity test. *Mutat. Res.*, 31, 347-364.
- Ames, B.N. (1979) Identifying environmental chemicals causing mutations and cancer. *Science*, 204, 587-593.
- Ashoor, S.H., Dietrich, R.A., Chu, F.S., and Pariza, M.W. (1980) Proline enhances mutagen formation in ground beef during frying. *Life Sci.*, 26, 1801.
- Baker, R., Arlauskas, A., Bonin, A. and Angus, D. (1982) Detection of mutagenic activity in human urine following fried pork or bacon meals. *Cancer Lett.*, 16, 81-89.
- Barnes, W.S., Maher, J.C. and Weisburger, J.H. (1983a) High pressure liquid chromatographic method for the analysis of 2-amino-3-methylimidazo[4,5-f]quinoline, a mutagen formed during the cooking of food. *J. Agric. Food Chem.*, 31, 883-886.
- Barnes, W.S., Spingarn, N.E., Gould, C.G., Vuolo, L.L., Wang, Y.Y. and Weisburger, J. H. (1983b) Mutagens in cooked foods: Possible consequence of the Maillard reaction. "The Maillard reaction in Foods and Nutrition." Wash. D.C. A.C.S. Symp. Ser., 215, 485-506.
- Barrington, P.J., Baker, R.S., Truswell, A.S., Bonin, A.M., Ryan, A.J. and Paulin, A.P. (1990) Mutagenicity of basic fractions derived from lamb and beef cooked by common household methods. *Food Chem. Toxicol.*, 28, 141-146.
- Becher, G., Knize, M.G., Nes, I.F. and Felton, J.S. (1988) Isolation and identification of mutagens from a fried Norwegian meat product. *Carcinogenesis*, 9, 247-253.
- Becher, G., Knize, M.G. and Felton, J.S. (1989) Identification and synthesis of new mutagens from a fried Norwegian meat product. *Var Foda*, 42, suppl. 2: 85-90.
- Berg, I., Overvik, E., Nord, C.-E. and Gustafsson, J.-A. (1988) Mutagenic activity in smoke formed during Broiling of lean pork at 200, 250 and 300°C. *Mutat. Res.*, 207, 199-204.
- Berg, I., Overvik, E. and Gustafsson, J.-A. (1990) Effect of cooking time on mutagen formation in smoke, crust and pan residue from pan-broiled pork. *Food Chem.*



- Toxicol.*, 28, 421-426.
- Bjeldanes, L. F. and Chew, H. (1979) Mutagenicity of 1,2-dicarbonyl compounds : maltol, kojic acid, diacetyl and related substances. *Mutat. Res.*, 67, 367-371.
- Bjeldanes, L.F., Morris, M.M., Felton, J.S., Healy, S.K., Stuermer, D.H., Berry, P., Timourian, H., Hatch, F.T. (1982a) Mutagens from the cooking of food. II. Survey by Ames/*Salmonella* test of mutagen formation in the major protein-rich foods of the American diet. *Food Chem. Toxicol.*, 20, 357-363.
- Bjeldanes, L.F., Morris, M.M., Felton, J.S., Healy, S.K., Stuermer, D.H., Berry, P., Timourian, H., Hatch, F.T. (1982b) Mutagens from the cooking of food. III. Secondary sources of cooked dietary protein. *Food Chem. Toxicol.*, 20, 365-369.
- Bjeldanes, L.F., Grose, K.R., Davis, P.H., Stuermer, D.H., Healy, S.K. and Felton, J. S. (1982c) An XAD-2 resin method for efficient extraction of mutagens from fried ground beef. *Mutat. Res.*, 105, 43-49.
- Bjeldanes, L.F., Morris, M.M., Timourian, H., Hatch, F.T. (1983) Effects of meat composition and cooking conditions on mutagenicity of fried ground beef. *J. Agric. Food Chem.*, 31, 18-21.
- Buttery, R.G., Ling, L.C., Teranishi, R. and Mon, T.R. (1977) Roasted lamb fat : basic volatile components. *J. Agric. Food Chem.*, 25, 1227-1280.
- Commoner, B., Vithayathil, A. J., Dolara, P., Nair, S., Madyastha, P. and Cuca, G. C. (1978) Formation of mutagens in beef and beef extract during cooking. *Science*, 201, 913-916.
- Coppola, E. D. (1984) Use of HPLC to monitor juice authenticity. *Food Technology*, 38, 88-91.
- Dolara, P., Commoner, B., Vithayathil, A.J., Cuca, G.C., Tuley, E., Madyastha, P., Nair, S. and Kriebel, D. (1979) The effect of temperature on the formation of mutagens in heated beef stock and cooked ground beef. *Mutat. Res.*, 60, 231-237.
- Felton, J. S., Healy, S. K., Stuermer, D. H., Berry, C., Timourian, H., Hatch, F.T., Morris, M. and Bjeldanes, L. F. (1981) Mutagens from the cooking of food. 1. Improved isolation and characterization of mutagenic fractions from cooked ground beef. *Mutat. Res.*, 88, 33-44.
- Felton, J.S., Knize, M.G., Wood, C., Wuebbles, B.J., Healy, S.K., Stuermer, D.H., Bjeldanes, L.F., Kimble, B.J. and Hatch, F.T. (1984) Isolation and characterization of new mutagens from fried ground beef. *Carcinogenesis*, 5, 95-102.

- Felton, J.S., Knize, M.G., Shen, N.H., Lewis, P.R., Andresen, B.D., Happe, J.A. and Hatch, F.T. (1986a) The isolation and identification of a new mutagen from fried ground beef : 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP). *Carcinogenesis*, 7, 1081-1086.
- Felton, J.S., Knize, M.G., Shen, N.H., Andresen, B.D., Bjeldanes, L.F. and Hatch, F. T. (1986b) Identification of the mutagen in cooked beef. *Environ. Health Perspect.*, 67, 17-24.
- Felton, J.S., Knize, M.G., Shen, N.H., Wu, R. and Becher, G. (1988) Mutagenic heterocyclic imidazoamines in cooked foods. In : King, C.M., Romano, L.J. and Schuetzle, D. (Eds.): " *Carcinogenic and Mutagenic Response to Aromatic Amines and Nitroarenes* ", Elsevier Science Publishing Co., Inc. Press, 73-85.
- Felton, J.S. and Knize, M.G. (1990a) Heterocyclic-amine mutagen/ carcinogens in foods , in : Cooper, C.S. and Grover, P.L. (Eds.), *Chemical Carcinogenesis and Mutagenesis I*, Handbook of Experimental Pharmacology, Vol. 94, Springer, Berlin, 471-502.
- Felton, J.S. and Knize, M.G. (1990b) New mutagens from cooked food, in: Pariza, M., Aeschbacher, H.U., Felton, J.S. and Sato, S. (Eds.), *Mutagens and Carcinogens in the Diet*, Wiley-Liss, New York, 19-38.
- Felton, J.S. and Knize, M.G. (1991) Occurrence, identification and bacterial Mutagenicity of heterocyclic amines in cooked food. *Mutation Res.*, 259, 205-217.
- Gazzani, G., Vagnrelli, P., Cuzzoni, M.T. and Mazza, P.G. (1987) Mutagenic activity of the Maillard reaction products of ribose with different amino acids. *J. Food Science*, 52, 757-760.
- Gomez-Arroyo, S. and Souza, V. (1985) *In vitro* and occupational induction of sister -chromatid exchange in human lymphocytes with furfuryl alcohol and furfural. *Mutation Res.*, 156, 233-243.
- Grivas, S., Nyhammar, T., Olsson, K. and Jagerstad, M. (1985a) Formation of a new mutagenic DiMeIQx compound in a model system by heating creatinine, alanine and fructose. *Mutat. Res.*, 151, 177-183.
- Grivas, S. and Nyhammar, T. (1985b) Analysis of mutagenic imidazo[4,5-*f*]quinolines and -quinoxalines (IQ compounds). Comparison of electrochemical and ultraviolet detection. *Mutat. Res.*, 142, 5-8.
- Grivas, S., Nyhammar, T., Olsson, K. and Jagerstad, M. (1986) Isolation and identifi-

- cation of the food mutagens IQ and MeIQx from a heated model system of creatinine, glycine and fructose. *Food Chem.*, 20, 127-136.
- Grose, K.R., Grant, J.L., Bjeldanes, L.F., Andresen, B.D., Healy, S.K., Lewis, P., Felton, J.S. and Hatch, F.T. (1986) Isolation of the carcinogen IQ from fried egg patties. *J. Agric. Food Chem.*, 3, 201-202.
- Gross, G.A., Philipposian, G. and Aeschbacher, H.U. (1989) An efficient and convenient method for the purification of mutagenic heterocyclic amines in heated meat products. *Carcinogenesis*, 10, 1175-1182.
- Gross, G.A. (1990) Simple methods for quantifying mutagenic heterocyclic aromatic amines in food products. *Carcinogenesis*, 11, 1597-1603.
- Hargraves, W.A. and Pariza, M.W. (1982) Purification and characterization of bacterial mutagens from commercial beef extracts and fried ground beef. *Proc. Am. Assoc. Cancer Res.*, 23, 92.
- Hargraves, W.A. and Pariza, M.W. (1983) Purification and mass spectral characterization of bacterial mutagens from commercial beef extract. *Cancer Res.*, 43, 1467-1472.
- Hatch, F.T., Knize, M.G. and Felton, J.S. (1991) Quantitative structure-activity relationships of heterocyclic amine mutagens formed during the cooking of food. *Environ. Mol. Mutagen.*, 17, 4-19.
- Hayatsu, H., Oka, T., Wakata, A., Ohara, Y., Hayatsu, T., Kobayashi, H. and Arimoto, S. (1983a) Adsorption of mutagens to cotton bearing covalently bound trisulfo-copper-phthalocyanine. *Mutat. Res.*, 119, 233-238.
- Hayatsu, H., Matsui, Y., Ohara, Y., Oka, T. and Hayatsu, T. (1983b) Characterization of mutagenic fractions in beef extract and in cooked ground beef. Use of Blue-cotton for efficient extraction. *Gann*, 74, 472-482.
- Hodge, J.E. and Rist, C.E. (1953) The Amadori rearrangement under new conditions and its significance for non-enzymatic browning reactions. *J. Am. Chem. Soc.*, 75, 316.
- Hong, J. and Richard, A.B. (1992) Effect of microwave heating on pyrazine formation in a model system. *J. Sci. Food Agri.*, 59, 283-289.
- Inamasu, T., Luks, H.J. and Weisburger, J.H. (1988) Comparison of XAD-2 column and Blue cotton batch techniques for isolation of metabolites of 2-amino-3-methylimidazo[4,5-f]quinoline. *Gann*, 79, 42-48.

- Jagerstad, M., Laser Reutersward, A., Oste, R., Dahlqvist, A., Grivas, S., Olsson, K. and Nyhammar, T. (1983a) Creatinine and Maillard reaction products as precursors of mutagenic compounds formed in fried beef. In *The Maillard Reaction in Food and Nutrition*. In: Waller, G.R. and Feather, M.S. (Eds.), Am. Chem. Soc., Wash. D.C., 507- 519.
- Jagerstad, M., Olsson, K., Grivas, S., Nyhammar, T., Olsson, K. and Dahlqvist, A. (1983b) Creatinine and Maillard reaction products as precursors of mutagenic compounds : effects of various amino acids. *Food Chem.*, 12, 255-264.
- Jagerstad, M., Olsson, K., Grivas, S., Negishi, C., Wakabayashi, K., Tsuda, M., Sato, S. and Sugimura, T. (1984) Formation of 2-amino-3,8-dimethylimidazo[4,5-f]-quinoxaline in a model system by heating creatinine, glycine and glucose. *Mutat. Res.*, 126, 239-244.
- Jagerstad, M., Grivas, S., Olsson, K., Laser Reutersward, A., Negishi, C. and Sato, S. (1986) Formation of food mutagens via Maillard reactions. *Genetic Toxicol. of the Diet*, Alan R. Liss, Inc., 155-167.
- Jagerstad, M., Skog, K., Grivas, S. and Olsson, K. (1990) Mutagens from model system. *Mutagens and Carcinogens in the Diet*, Wiley-Liss, Inc., 71-88.
- Jagerstad, M., Skog, K., Grivas, S. and Olsson, K. (1991) Formation of heterocyclic amines using model systems. *Mutat. Res.*, 259, 219-233.
- Jones, R.C. and Weisburger, J.H. (1989) Characterization of amino alkylimidazol-4-one mutagens from liquid-reflux models. *Mutat. Res.*, 222, 43-51.
- Kasai, H., Nishimura, S., Nagao, M., Takahashi, Y. and Sugimura, T. (1979) Fractionation of a mutagenic principle from broiled fish by high-pressure liquid chromatography. *Cancer Lett.*, 7, 343-348.
- Kasai, H., Yamaizumi, Z., Wakabayashi, K., Nagao, M., Sugimura, T., Yokoyama, S., Miyazawa, T., Spingarn, N.E., Weisburger, J.H. and Nishimura, S. (1980a) Potent novel mutagens produced by broiling fish under normal conditions. *Proc. Jpn. Acad.*, 56B, 278-283.
- Kasai, H., Nishimura, S., Wakabayashi, K., Nagao, M. and Sugimura, T. (1980b) Chemical synthesis of 2-amino-3-methylimidazo[4,5-f]quinoline (IQ), a potent mutagen isolated from broiled fish. *Proc. Jpn. Acad.*, 58, 382-384.
- Kasai, H., Yamaizumi, Z., Wakabayashi, K., Nagao, M., Sugimura, T., Yokoyama, S., Miyazawa, T. and Nishimura, S. (1980c) Structure and chemical synthesis of Me-

- IQ, a potent mutagen isolated from broiled fish. *Chem. Lett.*, 1391-1394.
- Kasai, H., Yamaizumi, Z., Nishimura, S., Wakabayashi, K., Nagao, M., Sugimura, T., Spingarn, N.E., Weisburger, J.H., Yokoyama, S. and Miyazawa, T. (1981a) A potent mutagen in broiled fish. Part 1., 2-amino-3-methyl-<sup>3</sup>H-imidazo[4,5-*f*]-quinoline. *J. Chem. Soc. Perkin I*, 2290-2293.
- Kasai, H., Yamaizumi, Z., Shiomi, T., Yokoyama, S., Miyazawa, T., Wakabayashi, K., Nagao, M., Sugimura, T. and Nishimura, S. (1981b) Structure of a potent mutagen isolated from fried beef. *Chem. Lett.*, 485-488.
- Kato, T., Kikugawa, K. and Hayatsu, H. (1986) Occurrence of the mutagens 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) and 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (4,8-DiMeIQx) in some Japanese smoked, dried fish products. *J. Agric. Food Chem.*, 34, 810-814.
- Kato, T., Ohgaki, H., Hasegawa, H., Sato, S., Takayama, S. and Sugimura, T. (1988) Carcinogenicity in rats of a mutagenic compound, 2-amino-3,8-dimethylimidazo [4,5-*f*] quinoxaline. *Carcinogenesis*, 9, 71.
- Kikugawa, K., Kato, T. and Hayatsu, H. (1986) The presence of 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline in smoked dry bonito (Katsuoobushi). *Gann*, 77, 99-102.
- Kikugawa, K. and Kato, T. (1987) Formation of mutagens, 2-amino-3,8-dimethylimidazo [4,5-*f*] quinoxaline (MeIQx) and 2-amino-3,4,8-trimethylimidazo [4,5-*f*] quinoxaline (4,8-DiMeIQx), in heated fish meats. *Mutat. Res.*, 179, 5-14.
- Knize, M.G., Andresen, B.D., Healy, S.K., Shen, N.H., Lewis, P.R., Bjeldanes, L.F., Hatch, F.T. and Felton, J.S. (1985) Effect of temperature, patty thickness and fat content on the production of mutagens in fried ground beef. *Food Chem. Toxicol.*, 23, 1035-1040.
- Knize, M.G., Happe, J.A., Healy, S.K. and Felton, J.S. (1987) Identification of the mutagenic quinoxaline isomers from fried ground beef. *Mutat. Res.*, 178, 25-32.
- Knize, M.G., Shen, N.H. and Felton, J.S. (1988a) The production of mutagens in foods. *Proc. Air Pollution Control Assoc.*, 3:1, 88-130.
- Knize, M.G., Shen, N.H. and Felton, J.S. (1988b) A comparison of mutagen production in fried ground chicken and beef: effect of supplemental creatine. *Mutagenesis*, 3, 503-508.
- Krone, C.A. and Iwaoka, W.T. (1983) Differences in observed mutagenicity associated

- with the extraction of mutagens from cooked fish. *J. Agric. Food Chem.*, 31, 428-431.
- Krone, C.A., Yeh, S.M.J. and Iwaoka, W.T. (1986) Mutagen formation during commercial processing of foods. *Environ. Health Perspect.*, 67, 75-88.
- Krone, C.A. and Iwaoka, W.T. (1987) Commercial food processing operations and mutagen formation. *J. of Food Protection.*, 50, 167-174.
- Larsen, K. (1972) Creatinine assay by a reaction-kinetic principle. *Clin. Chem. Acta.*, 41, 209.
- Laser Reutersward, A., Skog, K. and Jagerstad, M. (1987a) Effects of creatine and creatinine content on the mutagenic activity of meat extracts, bouillons and gravies from different sources. *Food Chem. Toxicol.*, 25, 747-754.
- Laser Reutersward, A., Skog, K. and Jagerstad, M. (1987b) Mutagenicity of pan-fried bovine tissues in relation to their content of creatine, creatinine monosaccharides and free amino acid. *Food Chem. Toxicol.*, 25, 755-762.
- Lee, H. and Lin, J. Y. (1981) Detection of mutagens in boiled pork extracts. *J. Chinese Biochem. Soci.*, 10, 71-75.
- Lee, H. and Tsai, S.J. (1991) Detection of IQ-type mutagens in canned roasted eel. *Food Chem. Toxicol.*, 29, 517-522.
- Lin, J.Y., Lee, H. and Huang, H.I (1982) Formation of mutagens in boiled pork extracts. *Food and Chemical Toxicol.*, 20, 531-533.
- Lijinsky, W. and Shubik, P. (1964) Benzo(a)pyrene and other polynuclear hydrocarbons in charcoal-broiled meat. *Science*, (Wash. D.C.), 145, 53-54.
- Macy, R.L., Naumann, H.D. and Bailey, M.E. (1964) Water-soluble flavor and odor precursors of meat. II. Effects of heating on amino nitrogen constituents and carbohydrates in lyophilized diffusates from aqueous extracts of beef, pork and lamb. *J. Food Sci.*, 29, 142-148.
- Manabe, S., Kurihara, N., Wada, O., Tohyama, K. and Aramaki, T. (1992) Formation of PhIP in a mixture of creatinine, phenylalanine and sugar or aldehyde by aqueous heating. *Carcinogenesis*, 13, 827-830.
- Maron, D.M. and Ames, B.N. (1983) Revised methods for the *Salmonella* mutagenicity test. *Mutat. Res.*, 113, 173-215.
- Masuda, Y., Mori, K. and Kuratsune, M. (1966) Polycyclic aromatic hydrocarbons in common Japanese foods. I. Broiled fish, roasted barley, shoyu and caramel. *Gann*

, 57, 133-142.

- Matsumoto, T., Yoshida, D., Mizusaki, S., and Okamoto, H. (1977) Mutagenic activity of amino acid pyrolysates in *Salmonella typhimurium* TA98. *Mutat. Res.*, 48, 279-286.
- Matsumoto, T., Yoshida, D. and Tomita, H. (1981) Determination of mutagens, amino- $\alpha$ -carbolines in grilled foods and cigarette smoke condensate. *Cancer Lett.*, 12, 105-110.
- Muramatsu, M. and Matsushima, T. (1985) Formation of MeIQx and 4,8-DiMeIQx by heating mixtures of creatinine, amino acids and monosaccharides. *Mutat. Res.*, 147, 266-267. (abstract)
- Murray, S., Gooderham, N.J., Boobis, A.R., Davies, D.S. (1988) Measurement of MeIQx and DiMeIQx in fried beef by capillary column gas chromatography electron capture negative ion chemical ionisation mass spectrometry. *Carcinogenesis*, 9, 321-325.
- Mussinan, C.J., Walradt, J.P. and Katz, I. (1973) Isolation and identification of pyrazines present in pressure-cooked beef. *J. Agric. Food Chem.*, 22, 827-830.
- Nagao, M., Honda, M., Seino, Y., Yahagi, T. and Sugimura, T. (1977) Mutagenicities of smoke condensates and the charred surface of fish and meat. *Cancer Lett.*, 2, 221-226.
- Nagao, M., Wakabayashi, K., Kasai, H., Nishimura, S. and Sugimura, T. (1981) Effect of methyl substitution on mutagenicities of 2-amino-3-methylimidazo[4,5-f]quinoline, isolated from broiled sardine. *Carcinogenesis*, 2, 1147-1149.
- Nagao, M., Sato, S. and Sugimura, T. (1983) Mutagens produced by heating foods. In: Waller, G.R. and Feather, M.S. (Eds.) *"The Maillard Reaction in Foods and Nutrition."*, Am. Chem. Soc., Wash. D.C., 215, 521-536.
- Negishi, C., Wakabayashi, K., Tsuda, M., Sato, S., Sugimura, T., Saito, H., Maeda, M. and Jagerstad, M. (1984) Formation of 2-amino-3,7,8-trimethylimidazo[4,5-f]quinoxaline, a new mutagen, by heating a mixture of creatinine, glucose and glycine. *Mutat. Res.*, 140, 55-59.
- Nes, I.F. (1986) Mutagen formation in fried meat emulsion containing various amounts of creatine. *Mutat. Res.*, 175, 145-148.
- Nyhammar, T., Grivas, S., Olsson, K. and Jagerstad, M. (1986a) Formation of 4,8-DiMeIQx from the model system fructose, alanine and creatinine. Comparison with

- the isomeric 5,8-DiMeIQx. *Mutat. Res.*, 174, 5-9.
- Nyhammar, T. (1986b) Studies on the Maillard reaction and its role in the Formation of Food Mutagens, Doctor Thesis, Swedish University of Agricultural Sciences, ISBN 91-576-2658-8.
- O'Brien, J. and Walker, R. (1988) Toxicological effects of dietary Maillard reaction products in the rat. *Food Chemical Toxicol.*, 26, 775-783.
- O'Brien, J. and Morrissey, P.A. (1989) Nutrition and toxicological aspects of the Maillard browning reaction in foods. *Critical Reviews in Food Science and Nutrition*, 28, 211-248.
- Ohagki, H., Kusama, K., Matsukura, N., Morino, K., Hasegawa, H., Sato, S., Takayama, S. and Sugimura, T. (1984) Carcinogenicity in mice of a mutagenic compound, 2-amino-3-methylimidazo[4,5-f]quinoline from broiled sardine, cooked beef extract. *Carcinogenesis*, 5, 921-924.
- Ohagki, H., Hasegawa, H., Suenaga, M., Kato, T., Sato, S., Takayama, S. and Sugimura, T. (1986) Induction of hepatocellular carcinoma and highly metastatic squamous cell carcinomas in the forestomach of mice by feeding 2-amino-3,4-dimethylimidazo[4,5-f]quinoline. *Carcinogenesis*, 7, 1889.
- Ohagki, H., Hasegawa, H., Suenaga, M., Sato, S., Takayama, S. and Sugimura, T. (1987) Carcinogenicity in mice of a mutagenic compound, 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx) from cooked foods. *Carcinogenesis*, 8, 665-668.
- Omura, H., Jahan, N., Shinohara, K. and Murakami, H. (1983) Formation of mutagens by the Maillard reaction. In: "*The Maillard Reaction in Foods and Nutrition*". Waller, G.R. and Feather, M.S. (eds.), Am. Chem. Soc, Wash. D.C., 537-563.
- Overvik, E., Nilsson, L., Fredholm, L., Levin, O., Nord, C.-E. and Gustafsson, J.-A. (1984) High mutagenic activity formed in pan-broiled pork. *Mutat. Res.*, 135, 149-157.
- Overvik, E., Nilsson, L., Fredholm, L., Levin, O., Nord, C.-E. and Gustafsson, J.-A. (1987) Mutagenicity of pan residues and gravy from fried meat. *Mutat. Res.*, 187, 47-53.
- Overvik, E., Kleman, M., Berg, I. and Gustafsson, J.-A. (1989) Influence of creatine, amino acids, water on the formation of the mutagenic heterocyclic amines found in cooked meat. *Carcinogenesis*, 10, 2293-2301.
- Overvik, E. and Gustafsson, J.-A. (1990) Cooked-food mutagens: current knowledge



- of formation and biological significance. *Mutagenesis*, 5, 437-446.
- Pariza, M.W., Ashoor, S.H., Chu, F.S. and Lund, D.B. (1979) Effects of temperature and time on mutagen formation in pan-fried hamburger. *Cancer Lett.*, 7, 63-69.
- Powrie, W.D., Wu, C.H., Rosin, M.P. and Stich, H.F. (1981) Clastogenic and mutagenic activities of Maillard reaction model systems. *J. Food Chem.*, 46, 1433-1438.
- Powrie, W.D., Wu, C.H. and Molund, V.P. (1986) Browning reaction system as sources of mutagens and antimutagen. *Environ. Health Perspect.*, 67, 47-54.
- Prieto, J.A., Collar, C. and Benedito de Barber, C. (1990) Reversed-phase high performance liquid chromatographic determination of biochemical changes in free amino acids during wheat flour mixing and bread baking. *J. Chromatogr. Science.*, 28, 572-577.
- Rappaport, S.M., McCartney, M.C. and Wei, E.T. (1979) Volatilization of mutagens from beef during cooking. *Cancer Lett.*, 8, 139-145.
- Shibamoto, T. and Bernhard, R.A. (1978) Formation of heterocyclic compounds from the reaction of *L*-rhamnose with ammonia. *J. Agric. Food Chem.*, 26, 183-187.
- Shibamoto, T. (1980a) Heterocyclic compounds found in cooked meats. *J. Agric. Food Chem.*, 28, 237-243.
- Shibamoto, T. (1980b) Mutagenicity of 1,5 (or 7)-dimethyl-2,3,6,7-tetrahydro-1H-bicyclopentapyrazine obtained from a cyclotene/NH<sub>3</sub> browning model system. *J. Agric. Food Chem.*, 28, 883-884.
- Shibamoto, T. (1982) Occurrence of mutagenic products in browning model systems. *Food Tech.*, Mar. 59-62.
- Shibamoto, T. (1983) Possible mutagenic constituents in nitrite-treated soy sauce. *Food Chem. Toxicol.*, 21, 745-747.
- Shibamoto, T. (1984) Ames mutagenicity tests of products from a heated potato-starch system. *Food Chem. Toxicol.*, 22, 119.
- Shibamoto, T. (1989) Genotoxicity testing of Maillard reaction products. In: *The Maillard Reaction in Aging, Diabets and Nutrition*, Alan, R. (Eds.), Liss Inc. 359-376.
- Shinohara, K., Jahan, N., Tanaka, M., Yamamoto, K., Wu, R.T., Murakami, H. and Omura, H. (1983) Formation of mutagens by amino-carbonyl reactions. *Mutat. Res.*, 122, 279-286.
- Shinohara, K., Kim, E.H. and Omura, H. (1986) Furan as the mutagens formed by amino

- carbonyl reactions. *Dev. Food Sci.*, 13, 353-361.
- Shioya, M., Wakabayashi, K., Sato, S., Nagao, M. and Sugimura, T. (1987) Formation of a mutagen 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) in cooked beef, by heating a mixture containing creatinine, phenylalanine and glucose. *Mutat. Res.*, 191, 133-138.
- Skog, K. and Jagerstad, M. (1990) Effects of monosaccharides and disaccharides on the formation of food mutagens in model systems. *Mutat. Res.*, 230, 263-272.
- Skog, K. and Jagerstad, M. (1991) Effects of glucose on the formation of PhIP in a model system. *Carcinogenesis*, 12, 2297-2300.
- Skog, K., Jagerstad, M. and Laser Reuterswärd, A. (1992) Inhibitory effect of carbohydrates on the formation of mutagens in fried beef patties. *Food Chem. Toxicol.*, 30, 681-688.
- Spingarn, N.E. and Weisburger, J.H. (1979a) Formation of mutagens in cooked food. 1. beef. *Cancer Lett.*, 7, 259-264.
- Spingarn, N.E. and Grivie, C.T. (1979b) Formation of mutagens in sugar-ammonia model systems. *J. Agric. Food Chem.*, 27, 1319-1321.
- Stich, H.F., Stich, W. and Rosin, M.P. (1980) Mutagenic activity of pyrazine derivatives: a comparative study with *Salmonella typhimurium*, *Saccharomyces cerevisiae* and Chinese hamster ovary cells. *Food Cosmet. Toxicol.*, 18, 581-584.
- Sugimura, T., Nagao, M., Kawachi, T., Honda, M., Yahagi, T., Seino, Y., Sato, S., Matsukura, N., Matsushima, T., Shirai, A., Sawamura, M. and Matsumoto, H. (1977a) Mutagen-carcinogens in food with special reference to highly mutagenic pyrolytic products in broiled foods. In: Hiatt, H.H., Watson, J.D., Winsten, J.A. (Eds.) *Origins of human cancer*. Cold Spring Harbor, New York, 1561-1577.
- Sugimura, T., Kawachi, T., Nagao, M., Yahagi, T., Seino, Y., Okamoto, T., Shudo, K., Kosuge, T., Tsuji, K., Wakabayashi, K., Iitaka, Y. and Itai, A. (1977b) Mutagenic principle(s) in tryptophan and phenylalanine pyrolysis products. *Proc. Jpn. Acad.*, 53, 58-61.
- Takahashi, M., Wakabayashi, K., Nagao, M., Yamaizumi, Z., Sato, S., Kinoshita, N., Tomita, I. and Sugimura, T. (1985a) Identification and quantification of 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (4,8-DiMeIQx) in beef extract. *Carcinogenesis*, 6, 1537-1539.
- Takahashi, M., Wakabayashi, K., Nagao, M., Yamamoto, M., Masui, T., Goto, T., Kinoshita, N.

- ,N., Tomita, I. and Sugimura, T. (1985b) Quantification of 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) and 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx) in beef extracts by liquid chromatography with electrochemical detection (LCEC). *Carcinogenesis*, 6, 1195-1199.
- Takahashi, M., Kinae, N., Tomita, I., Wakabayashi, K., Nagao, M., Sato, T. and Sugimura, T. (1986) Quantification of heterocyclic amines in cooked foods. *Mutat. Res.*, 164, 281-282.(abstract)
- Takayama, S., Nakatsura, T., Ohgaki, H., Sato, S. and Sugimura, T. (1984) Demonstration of carcinogenicity of F344 rats of 2-amino-3-methylimidazo[4,5-f]quinoline from broiled sardine, fried beef and beef extract. *Gann*, 75, 467-470.
- Talor, R.T., Fultz, E. and Knize, M.G. (1985) Mutagen formation on a model beef boiling system. III. Purification and identification of three heterocyclic amine mutagens- carcinogens. *J. Environ. Sci. Health*, A20, 135-148.
- Talor, R.T., Fultz, E. and Knize, M.G. (1986) Mutagen formation in a model beef supernatant fraction. IV. Properties of the system. *Environ. Health Perspect.*, 67, 59-74.
- Talor, R.T., Fultz, E., Knize, M.G. and Felton, J.S. (1987) Formation of the fried ground beef mutagens 2-amino-3-methylimidazo[4,5-f]quinoline(IQ) and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) from *L*-phenylalanine (Phe)+ creatinine(Cre)(or creatine). *Environ. Mutagen.*, 9 [suppl.8], 106.
- Talor, R.T., Fultz, E. and Knize, M.G. (1989) Mutagen formation in a model beef supernatant fraction: purification and identification of the major mutagenic activities produced by dry heating, in *Proc. Mutagens and Carcinogens in the Diet*, Wiley-Liss, Inc., 26.
- Tikkanen, L.M. (1991) Sources of Mutagenicity in cooked Finnish foods. *Food Chem. Toxicol.*, 29, 87-92.
- Tsuda, M., Takahashi, Y., Nagao, M., Hirayama, T. and Sugimura, T. (1980) Inactivation of mutagens from pyrolysates of tryptophan and glutamic acid by nitrite in acidic solution. *Mutat. Res.*, 78, 331-339.
- Tsuda, M., Negishi, C., Makino, R., Sato, S., Yamaizumi, Z., Hirayama, T. and Sugimura, T. (1985) Uses of nitrite and hypochlorite treatments in determination of the contributions of IQ-type and non IQ-type heterocyclic amines to the mutagenicities in crude pyrolyzed materials. *Mutat. Res.*, 147, 335-341.

- Turesky, R.J., Wishnok, J.S., Tannenbaum, S.R., Pfund, R.A. and Buchi, G.H. (1983) Qualitative and quantitative characterization of mutagens in commercial beef extract. *Carcinogenesis*, 4, 863-866.
- Turesky, R.J., Bur, H., Huynh-Ba, T., Aeschbacher, H.U. and Milon, H. (1988) Analysis of mutagenic heterocyclic amines in cooked beef products by high performance liquid chromatography in combination with mass spectrometry. *Food Chem. Toxicol.*, 26, 501-509.
- Uyeta, M., Kanada, T., Mazaki, M., Taue, S. and Takahashi, S. (1979) Assaying mutagenicity of food pyrolysis products using the Ames test. Naturally occurring carcinogens -mutagens and modulators of carcinogenesis. *Jpn. Sci. Soc.*, 169-176.
- Vahl, M., Gry, J. and Nielsen, P.A. (1988) Mutagens in fried pork and the influence of the frying temperature. *Mutat. Res.*, 203, 239.(abstract)
- Voogd, C.E., Van der Stel, J.J. and Jacobs, J.J.A.A. (1979) The mutagenic action of nitroimidazoles. IV. A comparison of the mutagenic action of several nitroimidazoles and some imidazoles. *Mutat. Res.*, 60, 207-209.
- Yamaguchi, K., Shudo, K., Okamoto, T., Sugimura, T. and Kosuga, T. (1980a) Presence of 2-aminodipyrido[1,2-*a*:3',2'-*d*]imidazole in broiled cuttlefish. *Gann*, 71, 743-744.
- Yamaguchi, K., Shudo, K., Okamoto, T., Sugimura, T. and Kosuga, T. (1980b) Presence of 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole in broiled beef. *Gann*, 71, 745-746.
- Yamaizumi, Z., Shiomi, T., Kasai, H., Nishimura, S., Takahashi, Y., Nagao, M. and Sugimura, T. (1980) Detection of potent mutagens, Trp-P-1 and Trp-P-2, in broiled fish. *Cancer Lett.*, 9, 75-83.
- Yamaizumi, Z., Kasai, H., Nishimura, S., Edmonds, C.G. and McCloskey, J.A. (1986) Stable isotope dilution quantification of mutagens in cooked foods by combined liquid chromatography-thermospray mass spectrometry. *Mutat. Res.*, 173, 1-7.
- Yamamoto, T., Tsuji, K., Kosuge, T., Okamoto, T., Shudo, K., Takeda, K., Iitaka, Y., Yamaguchi, K., Seino, Y., Yahagi, T., Nagao, M. and Sugimura, T. (1978) Isolation and structure determination of mutagenic substances in *L*-glutamic acid pyrolysate. *Proc. Jpn. Acad.*, 54B, 248-250.
- Yoshida, D., Matsumoto, T., Yoshimura, R. and Matsuzaki, T. (1978) Mutagenicity

- of amino-alpha-carbolines in pyrolysis products of soybean globulin. *Biochem. Biophys. Res. Comm.*, 83, 915-920.
- Yoshida,D. and Okamoto,H.(1980) Formation of mutagens by heating the aqueous solution of amino acids and some nitrogenous compounds with addition of glucose. *Agric Biol. Chem.*, 44, 2521-2522.
- Yoshida,D.and Fukuhara,Y.(1982) Formation of mutagens by heating creatine and amino acids. *Agric Biol. Chem.*, 46, 1069-1070.
- Yoshida,D., Saito,Y.and Mizusaki,S. (1984) Isolation of 2-amino-3-methylimidazo[4,5-f]quinoline as mutagen from the heated product of a mixture of creatine and proline. *Agric. Biol. Chem.*, 48, 241-243.
- Zhang,X.M., Wakabayashi,K., Liu,Z.C., Sugimura,T. and Nagao,M. (1988) Mutagenic and carcinogenic heterocyclic amines in Chinese cooked foods. *Mutat. Res.*, 201, 181-188.

染。圖表

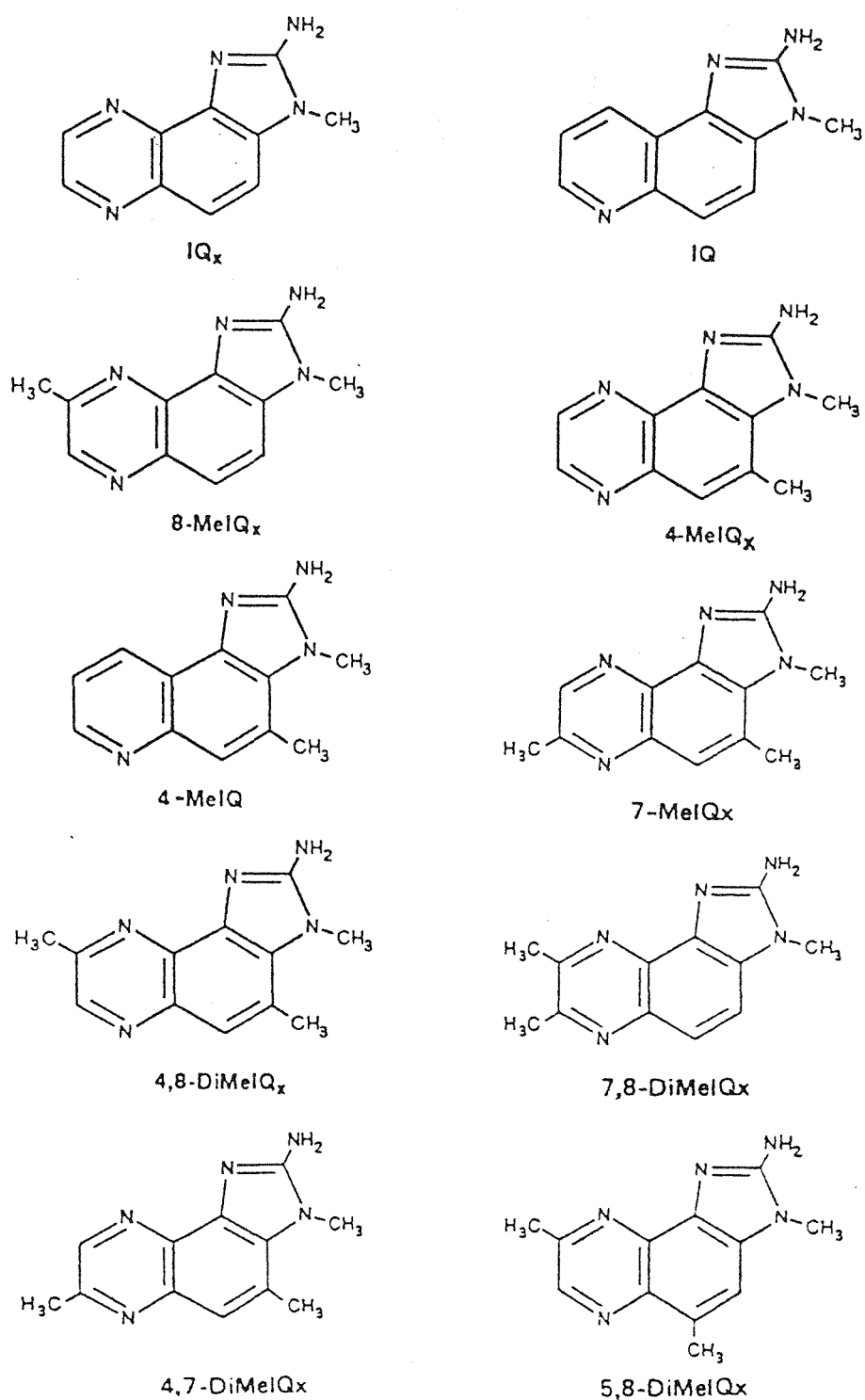
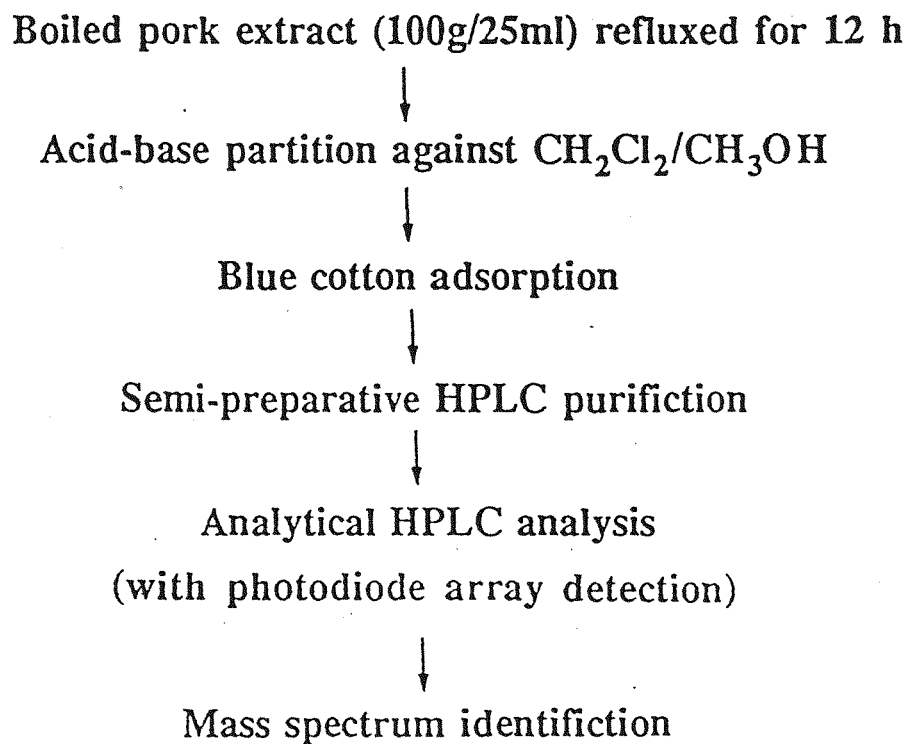


Fig.1 Chemical structure of the 10 mutagens of quinoline or quinoxaline.



**Fig. 2** The scheme of extraction and purification of heterocyclic amines from boiled pork extracts.

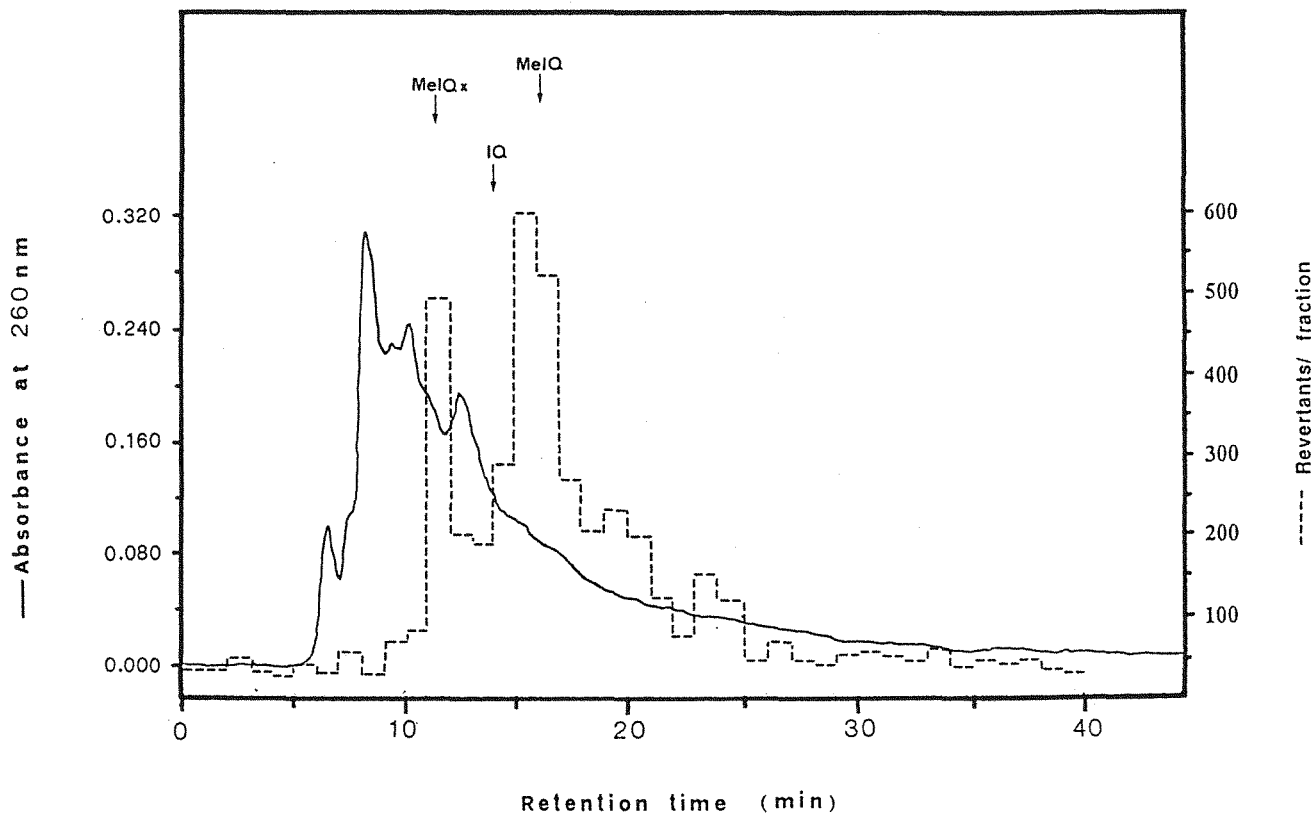


Fig. 3 The elution profile of the mutagens in boiled pork extract by semi-preparative HPLC on an ODS column. The mutagenicity of 1-min fraction was tested using *S. typhimurium* TA98 with S9 mix.



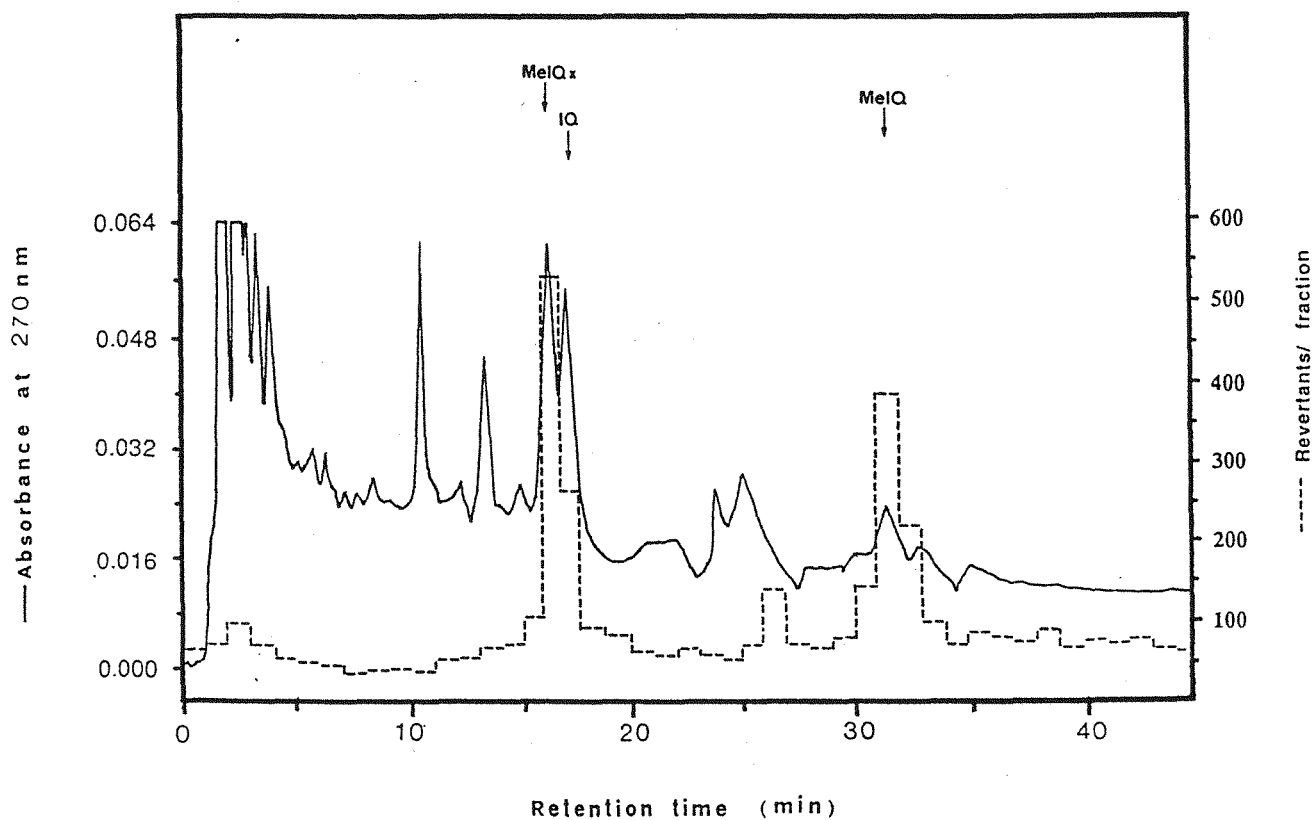
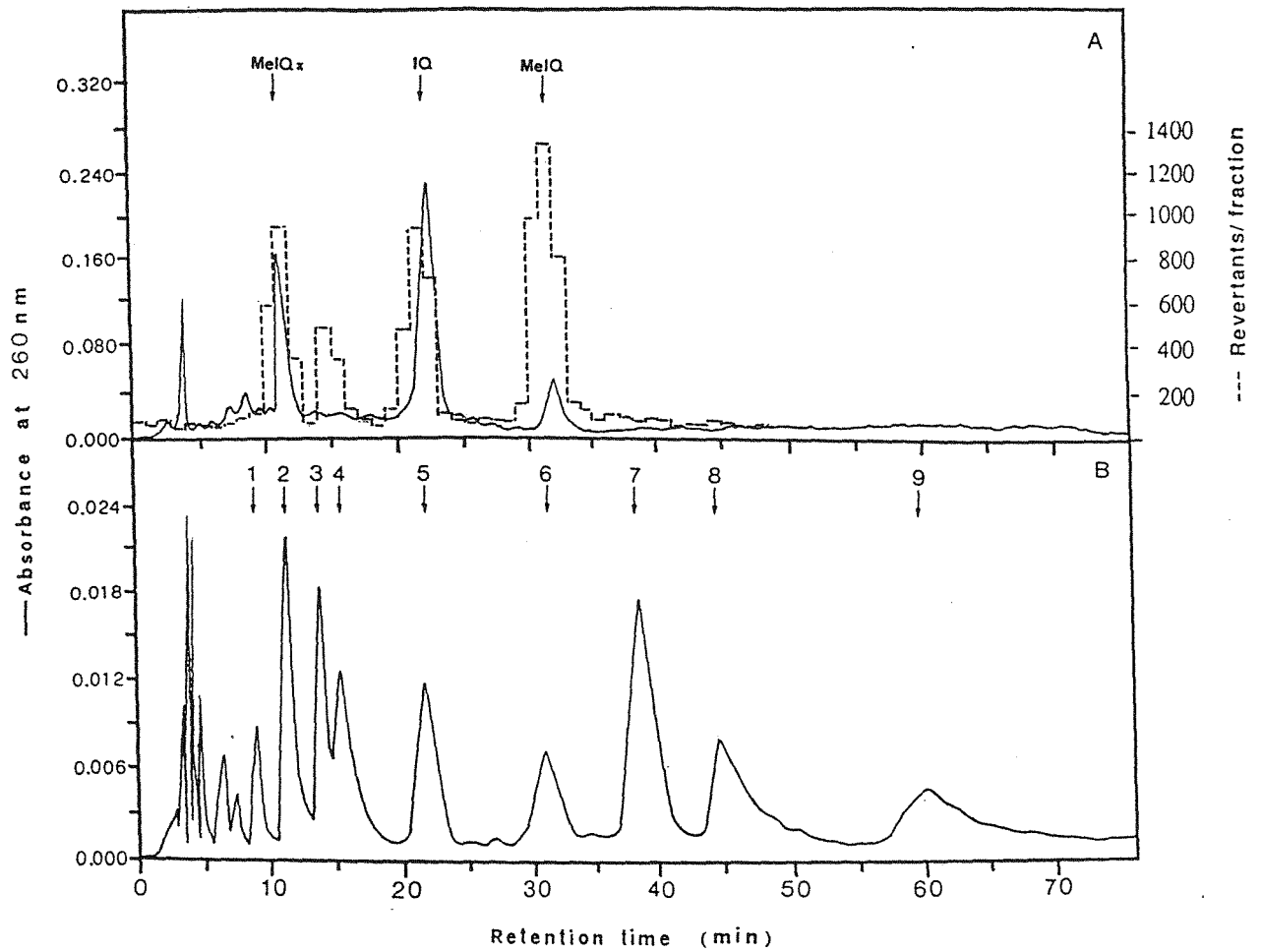


Fig. 4 The elution profile of the mutagens in boiled pork extracts by HPLC on an analytical Nucleosil 5 C<sub>18</sub> column. The mutagenicity of 1-min fraction was tested using *S. typhimurium* TA98 with S9 mix.

**Table 1 The strain specificity and nitrite treatment of mutagens in boiled pork extracts fractionated by HPLC.**

Test samples	Strain specificity		Nitrite treatment *	
	TA98	TA100	Before	After
HPLC mutagenic				
fraction 16	187±11	94±7 (2.7)	198±13	195±9 (98.5)
fraction 31	178±10	98±6 (5.8)	171±9	162±8 (94.7)
Positive control				
Trp-p-2	2663±122	117±10 (1.0)	2621±136	436±19 (16.6)
MeIQx	738±28	107±8 (2.4)	733±24	723±25 (98.6)
MeIQ	4511±255	342±29 (5.6)	4476±113	4295±133(96.0)
Negative control				
(-S9)	26±3	71±8		
(+S9)	40±4	90±10		

\*The procedure used for nitrite treatment is the same as that described by Tsuda *et. al.* (1985). The doses of test compounds used were about 0.35 g /plate boiled pork juice extract and 10 ng/plate standard. Values are expressed as means±SD.



**Fig. 5** Elution profiles of the mutagens in boiled pork extract (A) and authentic heterocyclic amines (B) by HPLC on an analytical Nucleosil 5CN column. The mutagenicity of 1-min fraction was tested using *S. typhimurium* TA98 with S9 mix. 1=DMIP; 2=MeIQx; 3=7,8-DiMeIQx; 4=4,8-DiMeIQx; 5=IQ; 6=MeIQ; 7=PhIP; 8=Trp-P-1; 9=Trp-P-2.

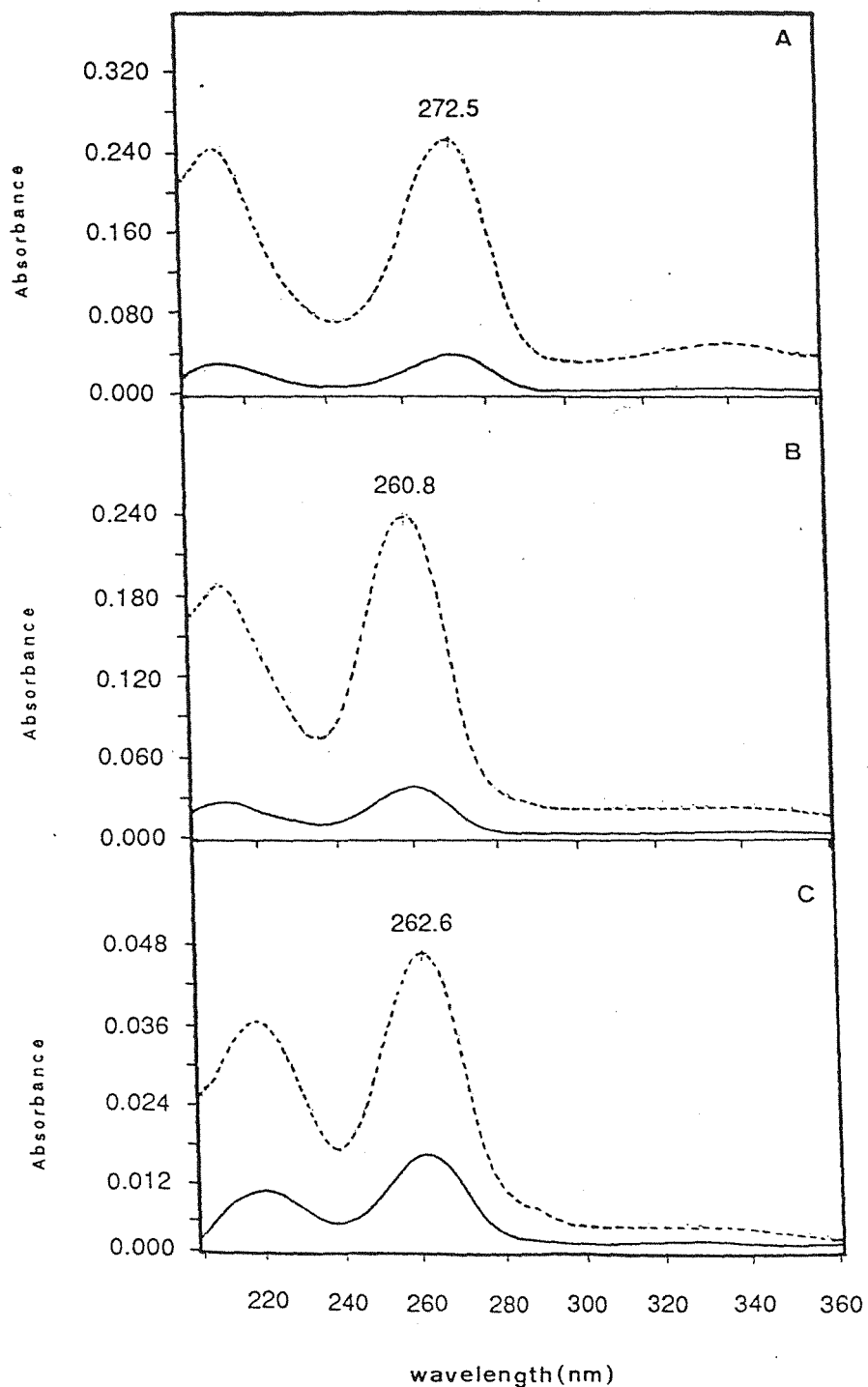


Fig. 6 Ultraviolet absorbance spectra of purified fraction of boiled pork extracts (---) and authentic standard compounds (—), MeIQx(A), IQ(B) and MeIQ(C) obtained from photodiode array detector by HPLC.

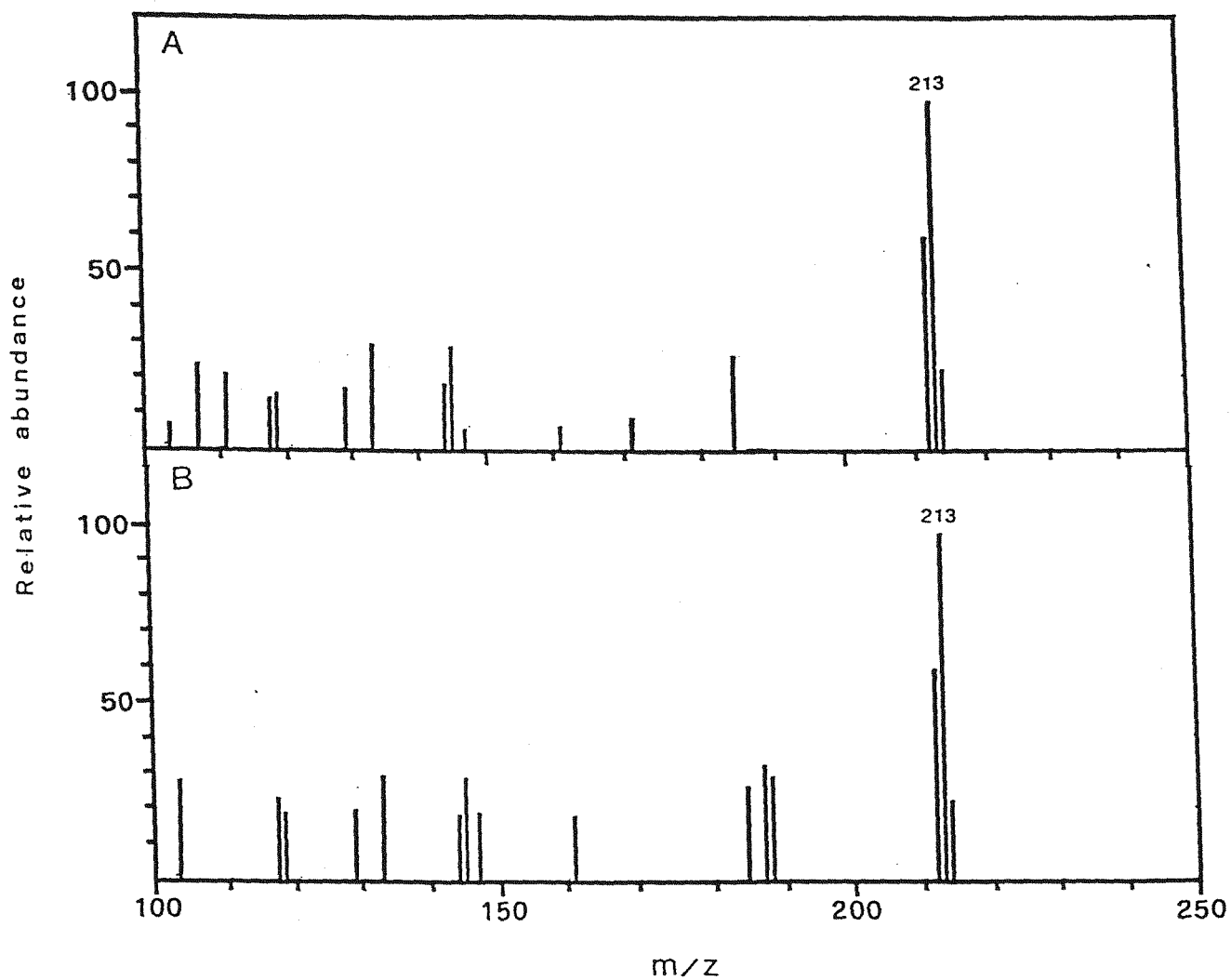


Fig. 7 Mass spectra of authentic MeIQx (A) and MeIQx in boiled pork extracts (B). Fragments occurred at less than  $m/z$  100 were not shown.

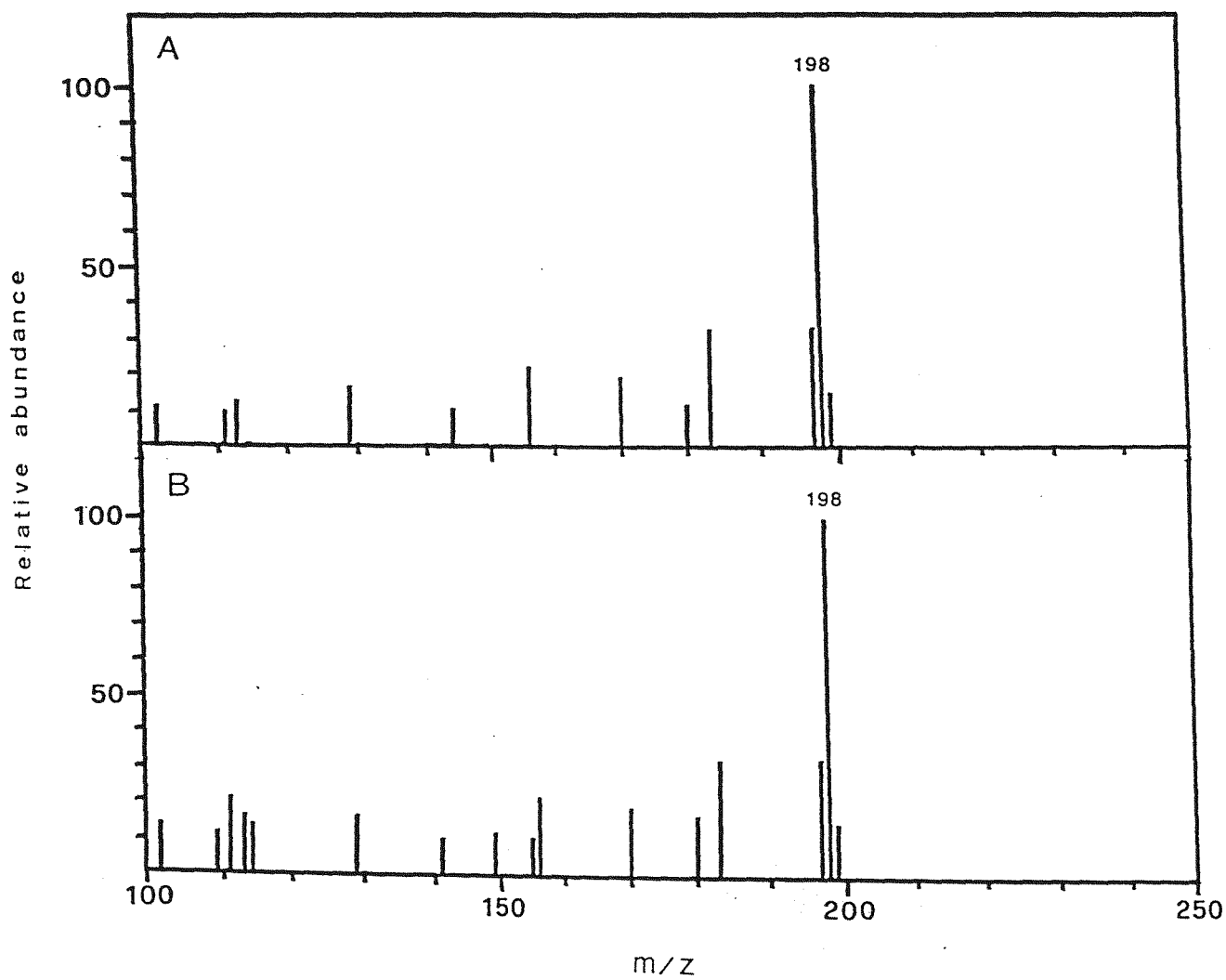


Fig. 8 Mass spectra of authentic IQ (A) and IQ in boiled pork extracts (B). Fragments occurred at less than m/z 100 were not shown.

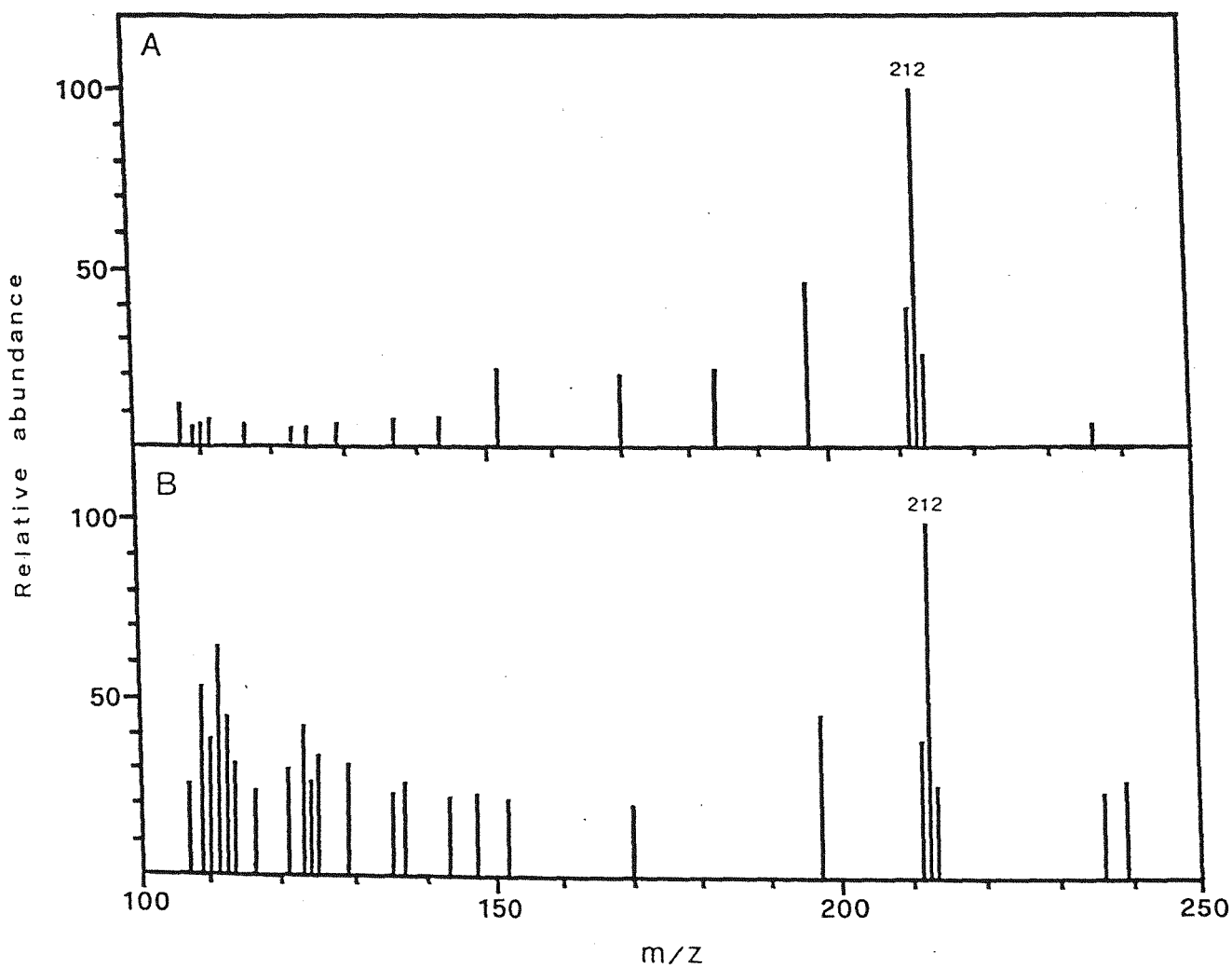


Fig. 9 Mass spectra of authentic MeIQ (A) and MeIQ in boiled pork extracts (B). Fragments occurred at less than  $m/z$  100 were not shown.

**Table 2** Distribution of mutagenic activity and amounts of IQ-type mutagens in boiled pork extracts.

Mutagen	Net revertants <sup>a</sup> /gE <sup>b</sup>	% of Total mutagenic activity	Amount (ng/g) <sup>c</sup>
MeIQx	303	19.0	4.1
IQ	437	27.3	3.7
MeIQ	548	34.3	1.2
DiMeIQx	151	9.4	—

<sup>a</sup> The net revertants determined from the mutagenicity deducted 2-fold spontaneous revertants in HPLC fractionation.

<sup>b</sup> gE : equivalent amount of 1 g wet meat.

<sup>c</sup> The amount of IQ-type mutagen is determined by HPLC.

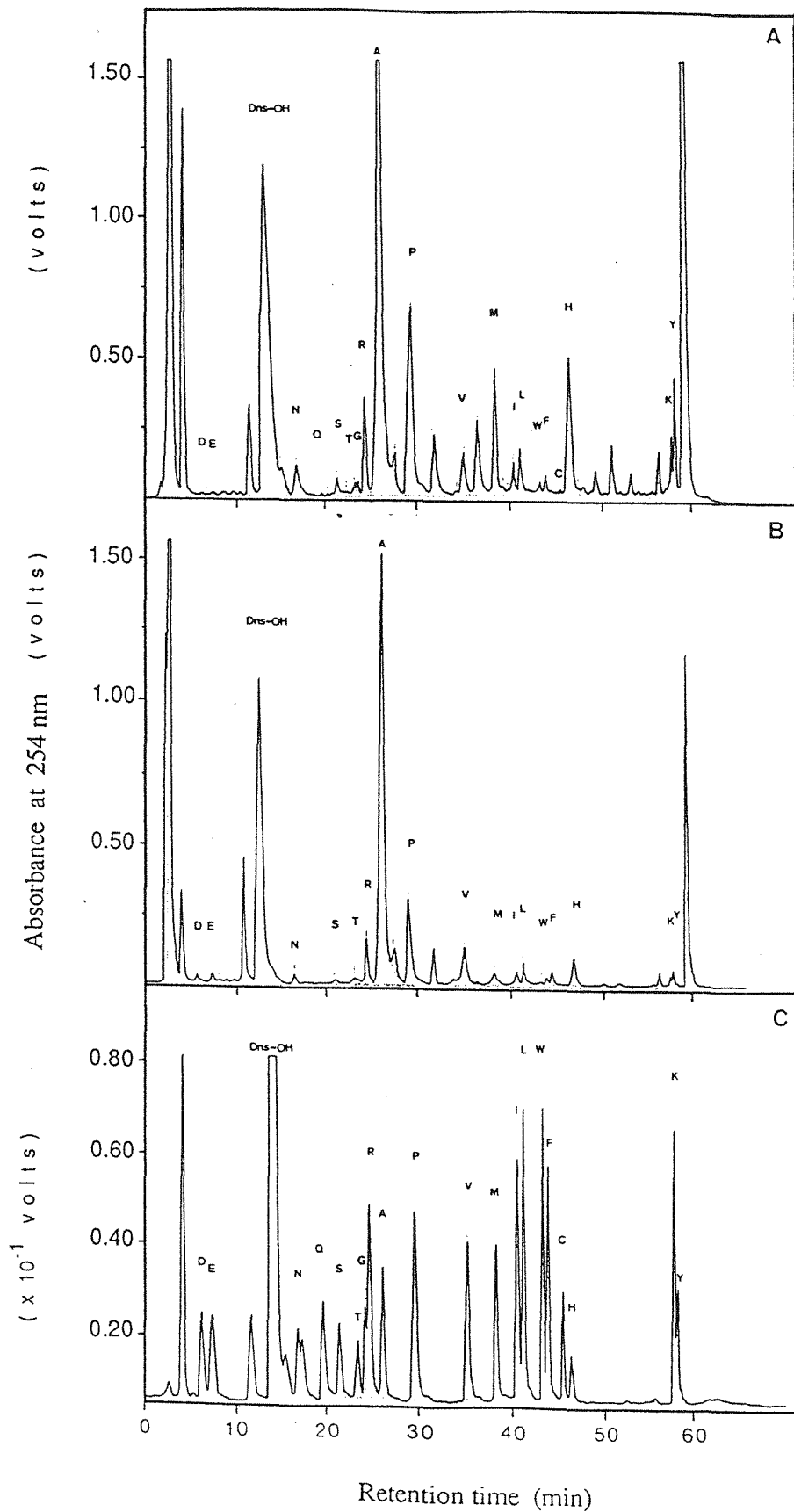


**Table 3 Comparison of amount and source of mutagens identified in heated foods and food extracts.**

Compound	Food	Amounts (ng/g)	Reference
MeIQx	Fried ground beef	0.45	Hargraves and Pariza(1983)
	Fried ground beef	0.1	Felton <i>et al.</i> (1984)
	Fried ground beef	1.3 -2.4	Murray <i>et al.</i> (1988)
	Smoked dried mackerel	0.8	Kato <i>et al.</i> (1986)
	Canned roasted eel	1.1	Lee and Tsai (1991)
	Food grade beef extract	28	Hargraves and Pariza(1983)
	Food grade beef extract	3.1	Takahashi <i>et al.</i> (1985b)
	Boiled pork extract	4.1	Present data
IQ	Broiled sardine	20	Kasai <i>et al.</i> (1981)
	Fried ground beef	0.6	Barnes <i>et al.</i> (1983)
	Fried ground beef	0.02	Felton <i>et al.</i> (1984)
	30-h boiled beef extract	0.5	Taylor <i>et al.</i> (1985)
	Broiled beef	0.5	Yamaizumi <i>et al.</i> (1986)
	Broiled sardine	4.9	Yamaizumi <i>et al.</i> (1986)
	Broiled salmon (skin)	1.1 -1.7	Yamaizumi <i>et al.</i> (1986)
	Broiled salmon (flesh)	0.3 -1.8	Yamaizumi <i>et al.</i> (1986)
	Boiled pork extract	3.7	Present data
MeIQ	Fried ground beef	< 0.1	Felton <i>et al.</i> (1986)
	Broiled sardine	16.6	Yamaizumi <i>et al.</i> (1986)
	Broiled salmon (skin)	1.5 -3.1	Yamaizumi <i>et al.</i> (1986)
	Broiled salmon (flesh)	0.6 -2.8	Yamaizumi <i>et al.</i> (1986)
	Boiled pork extract	1.2	Present data

**Table 4. Occurrence of heterocyclic amines identified in heat-processed foods and food extracts.**

Heated product	Compound identified	Reference	
Cuttlefish	Glu-P-2	Yamaguchi <i>et al.</i> (1980a)	
Sardines	Trp-P-1, Trp-P-2	Yamaizumi <i>et al.</i> (1980)	
	IQ, MeIQ	Kasai <i>et al.</i> (1980c)	
	IQ	Kasai <i>et al.</i> (1981a)	
Salmon	IQ, MeIQ	Yamaizumi <i>et al.</i> (1986)	
Mackerel	MeIQx, 4,8-DiMeIQx	Kato <i>et al.</i> (1986)	
Fish products	MeIQx	Kikugawa <i>et al.</i> (1986)	
	MeIQx, 4,8-DiMeIQx	Kikugawa and Kato (1987)	
Egg patties	IQ	Grose <i>et al.</i> (1986)	
Beef, chicken	A $\alpha$ C, MeA $\alpha$ C	Matsumoto <i>et al.</i> (1981)	
Beef	Trp-P-1	Yamaguchi <i>et al.</i> (1980b)	
	MeIQx	Kasai <i>et al.</i> (1981b)	
	MeIQx	Hargraves and Pariza (1983)	
	MeIQx	Felton <i>et al.</i> (1984)	
	PhIP	Felton <i>et al.</i> (1986a)	
	MeIQx, IQ, MeIQ, DiMeIQx, TMIP, PhIP	Felton <i>et al.</i> (1986b)	
	MeIQx, DiMeIQx	Murray <i>et al.</i> (1988)	
	IQ, MeIQ, MeIQx, 4,8-DiMeIQx	Knize <i>et al.</i> (1987)	
	IQ, MeIQx, 4,8-DiMeIQx	Turesky <i>et al.</i> (1988)	
	Beef extract	IQ, MeIQx	Turesky <i>et al.</i> (1983)
		IQ, MeIQx	Hayatsu <i>et al.</i> (1983a)
		IQ, MeIQ, MeIQx	Hargraves and Pariza (1983)
		4,8-DiMeIQx	Takahashi <i>et al.</i> (1985a)
IQ, MeIQx		Takahashi <i>et al.</i> (1985b)	
Fried pork	MeIQx, IQ, MeIQ, DiMeIQx, PhIP	Vahl <i>et al.</i> (1988) (abstract)	
	MeIQx, IQ, MeIQ, DiMeIQx	Present data	



**Fig. 10** The chromatography of dansyl-amino acid (Dns-AA) from standards (C) and boiled pork extracts before (A) or after (B) 12 h-boiling process analyzed by HPLC with UV detector.

**Table 5 Comparison of free amino acids concentration in pork extracts before and after 12h-boiling process.**

Amino acid	Amounts ( $\mu\text{mol/g}$ pork)			Decreased % <sup>b</sup>
	Boiled before	Boiled after	Decreased <sup>a</sup>	
Ala (A)	24.54	17.83	6.71	20.13
His (H)	15.81	2.88	12.93	38.79
Pro (P)	5.64	2.06	3.58	10.74
Met (M)	2.58	0.02	2.56	7.68
Asn (N)	2.39	0.87	1.52	4.56
Tyr (Y)	1.79	0.30	1.49	4.47
Arg (R)	1.64	0.81	0.83	2.49
Ser (S)	1.10	0.19	0.91	2.73
Val (V)	1.09	0.34	0.75	2.25
Thr (T)	0.71	0.69	0.02	0.06
Leu (L)	0.69	0.36	0.33	0.99
Gly (G)	0.55	trace	0.55	1.65
Lys (K)	0.48	0.13	0.35	1.05
Ile (I)	0.47	0.27	0.20	0.60
Phe (F)	0.30	0.19	0.11	0.33
Trp (W)	0.20	0.08	0.12	0.36
Glu (E)	0.11	0.29	-0.18	0.54
Asp (D)	0.08	0.22	-0.14	0.42
Gln (Q)	0.03	trace	0.03	0.09
Cys (C)	0.02	trace	0.02	0.06
Total	60.22	27.53	32.69	—

<sup>a</sup> Decreased = The amount of amino acid boiled before — the amount of amino acid boiled after.

<sup>b</sup> Decreased % = each amino acid decreased / total amino acid decreased.

**Table 6** Effects of single amino acid on the mutagenicity of boiled pork extracts with *Salmonella typhimurium* TA98 in the presence of S9 mix.

Amino acid addition (2.0 mmol)	Mutagenic ratio of crude reaction mixture *
Ala	14.7
Tyr	13.4
Gly	8.9
Asn	6.7
Leu	3.4
Pro	2.7
Val	1.7
Lys	1.7
His	1.4
Ser	1.4
Arg	1.0
Met	0.8

Data was abstracted from previous study ( Lee *et al.* unpublished data, 1991).

\* Total revertants of BPE adding amino acid / total revertants of BPE alone.

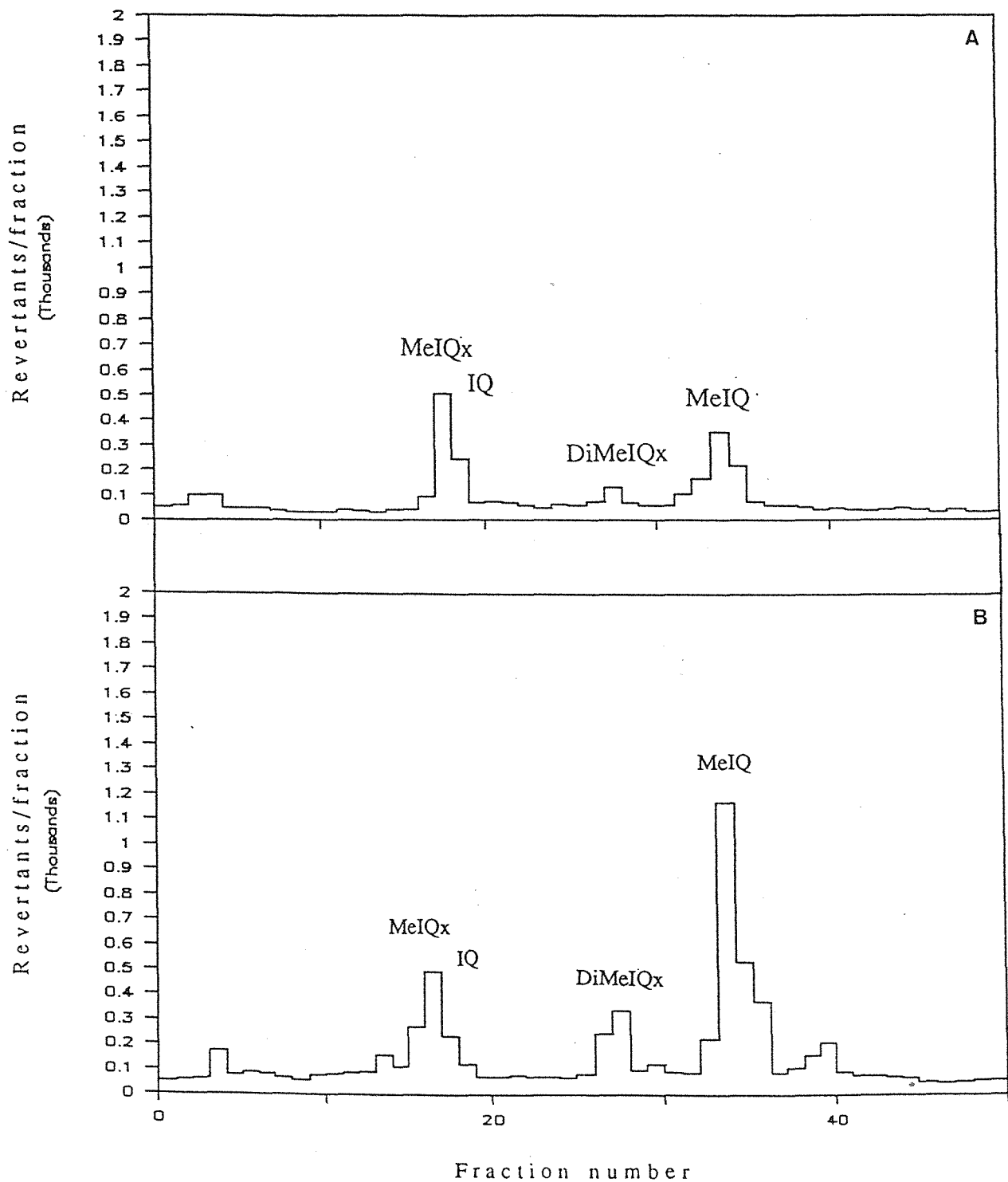


Fig. 11 The mutagenic profiles of analytic HPLC fractionation of boiled pork extracts (A) and with the addition of 2.0 mmol alanine (B).

**Table 7** Effects of the addition of alanine on the mutagen formation of boiled pork extracts by HPLC fractionation. The mutagenicity of HPLC elutions was tested with *S. typhimurium* TA98 in the presence of S9 mix.

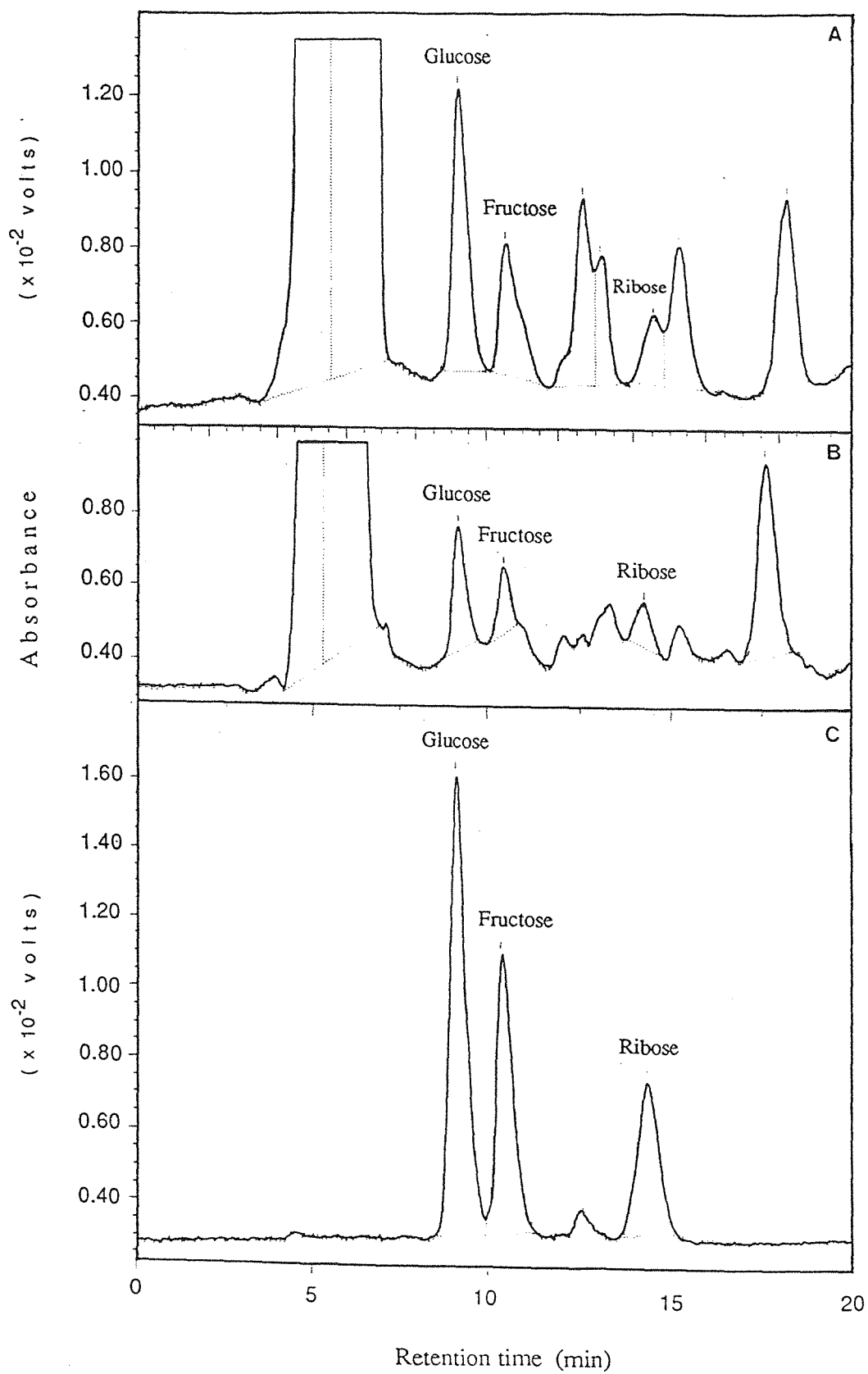
Model	Net revertants / fraction (ratio to BPE)			
	Unknown	MeIQx, IQ	DiMeIQx	MeIQ
BPE	75	677	86	606
BPE+alanine	97 (1.29)	891 (1.32)	449 (5.22)	1945 (3.21)

BPE: boiled pork extracts.

2.0 mmol alanine was added to the boiled pork model system.

ND: not detected.

The mutagenicity was tested with *Salmonella typhimurium* TA98 in the presence of S9 mix and 13 percent of total basic extracts were used in the mutagenicity testing.



**Fig. 12** The chromatography of monosaccharides from standards (C) and boiled pork extracts before (A) or after (B) 12 h-boiling process analyzed by HPLC with RI detector.



**Table 8** The amounts of chemical components in pork extracts before and after 12h-boiling process.

Component	Amounts ( $\mu\text{mol/g}$ pork)			Decreased ratio <sup>c</sup> (%)
	Boiled before	Boiled after	Decreased <sup>d</sup>	
Glucose <sup>a</sup>	0.89	0.41	0.48	53.93
Fructose <sup>a</sup>	0.85	0.64	0.21	24.71
Ribose <sup>a</sup>	0.54	0.30	0.24	44.44
Total amino acid <sup>a</sup>	60.22	27.53	32.69	54.28
Creatinine <sup>b</sup>	18.42	7.37	11.05	59.99

<sup>a</sup> Calculated from the calibration curve of HPLC quantification.

<sup>b</sup> Calculated by Jaffe kinetic method.

<sup>c</sup> Decreased ratio = component decreased amount / component boiled before amount.

<sup>d</sup> Decreased = The amount of component boiled before — the amount of component boiled after.

**Table 9** Effects of some components added to the boiled pork extracts model systems on the mutagenicity in *Salmonella typhimurium* TA98 with S9 mix.

Models	Total net revertants ( $\times 10^5$ )	Mutagenic ratio of basic extract
BPE <sup>a</sup>	1.58	—
BPE+glucose (2.0mmol)	4.28	2.71
BPE+ribose (2.0mmol)	10.63	6.73
BPE+alanine (2.0mmol)	4.78	3.03
BPE+creatinine (17.5mmol)	7.91	5.01
BPE+tetrahydrothiophen-3-one (5.0mmol)	6.43	4.07
H <sub>2</sub> O <sup>b</sup> +4cpds <sup>c</sup>	17.18	10.87
BPE +4cpds	35.45	22.44

All revertants represent total counts, corrected for spontaneous mutants. Spontaneous revertants of *S. typhimurium* TA98 were  $24 \pm 3$  (-S9) and  $45 \pm 5$  (+S9) respectively.

The amount of <sup>a</sup>BPE (boiled pork extracts) and <sup>b</sup>H<sub>2</sub>O were equal to 25ml pork juice / 100g pork and 25ml H<sub>2</sub>O, respectively.

<sup>c</sup> 4cpds : ribose (2.0mmol) + alanine (2.0mmol) + creatinine (17.5mmol) + tetrahydrothiophen-3-one (5.0mmol)

All model systems were boiled under reflux for 12 h at ca. 102°C.

**Table 10. Food mutagens isolated from model system of creatin(in)e and amino acids with or without sugar.**

Mutagen	Precursors Amino acid/ Creatin(in)e/ Sugar	Heating conditions	References
MeIQx	Glycine/ Creatinine / Glucose	DEG-H <sub>2</sub> O <sup>a</sup>	Jagerstad <i>et al.</i> (1984)
	Alanine/ Creatinine / Glucose	DEG-H <sub>2</sub> O <sup>b</sup>	Muramatsu <i>et al.</i> (1985)
	Alanine/ Creatinine / Ribose	DEG-H <sub>2</sub> O <sup>b</sup>	Muramatsu <i>et al.</i> (1985)
	Lysine/ Creatinine / Ribose	DEG-H <sub>2</sub> O <sup>b</sup>	Muramatsu <i>et al.</i> (1985)
	Glycine/ Creatinine / Fructose	DEG-H <sub>2</sub> O <sup>a</sup>	Grivas <i>et al.</i> (1986)
	Threonine/Creatinine/Glucose	DEG-H <sub>2</sub> O <sup>a</sup>	Jagerstad <i>et al.</i> (1986)
	Tyrosine/ Creatinine / -	Dry <sup>c</sup>	Overvik <i>et al.</i> (1989)
	Alanine / Creatinine / -	Dry <sup>c</sup>	Overvik <i>et al.</i> (1989)
	Serine / Creatinine / -	Dry <sup>c</sup>	Overvik <i>et al.</i> (1989)
IQ	Proline / Creatine / -	Dry <sup>d</sup>	Yoshida <i>et al.</i> (1984)
	Glycine/ Creatinine / Fructose	DEG-H <sub>2</sub> O <sup>a</sup>	Grivas <i>et al.</i> (1986)
	Serine / Creatinine / -	Dry <sup>c</sup>	Knize <i>et al.</i> (1988a)
	Phenyl -alanine/Creatinine/ Glucose	Dry <sup>c</sup>	Talor <i>et al</i> (1987)
	Phenyl -alanine/Creatinine/ -	Dry <sup>c</sup>	Talor <i>et al</i> (1987)
MeIQ	Alanine/ Creatinine / Fructose	DEG-H <sub>2</sub> O <sup>a</sup>	Grivas <i>et al.</i> (1985)
4,8-DiMeIQx	Alanine/ Creatinine / Fructose	DEG-H <sub>2</sub> O <sup>a</sup>	Grivas <i>et al.</i> (1986)
	Alanine/ Creatinine / Glucose	DEG-H <sub>2</sub> O <sup>b</sup>	Muramatsu <i>et al.</i> (1985)
	Threonine/Creatinine/Glucose	DEG-H <sub>2</sub> O <sup>a</sup>	Jagerstad <i>et al.</i> (1986)
7,8-DiMeIQx	Glycine/ Creatinine / Glucose	DEG-H <sub>2</sub> O <sup>a</sup>	Negishi <i>et al.</i> (1984)

<sup>a</sup> Diethylene glycol/Water mixture heated for 2 h at 128°C.

<sup>b</sup> Diethylene glycol/Water mixture heated for 3 h at 125°C.

<sup>c</sup> Dry heated for 1 h at 200°C.

<sup>b</sup> Dry heated for 1 h at 180°C.

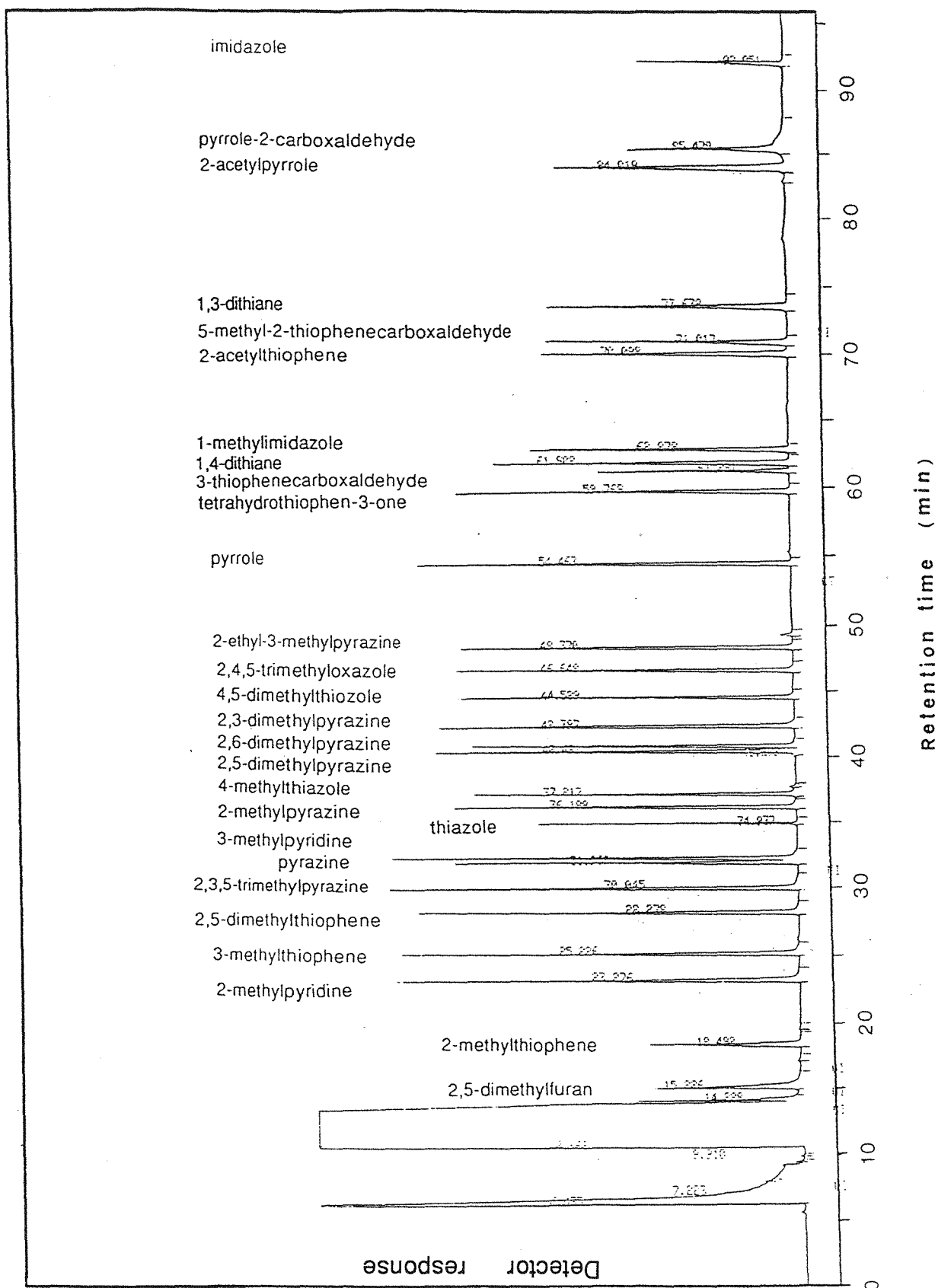


Fig. 13 Gas chromatography of Maillard reaction product (MRP) standards.

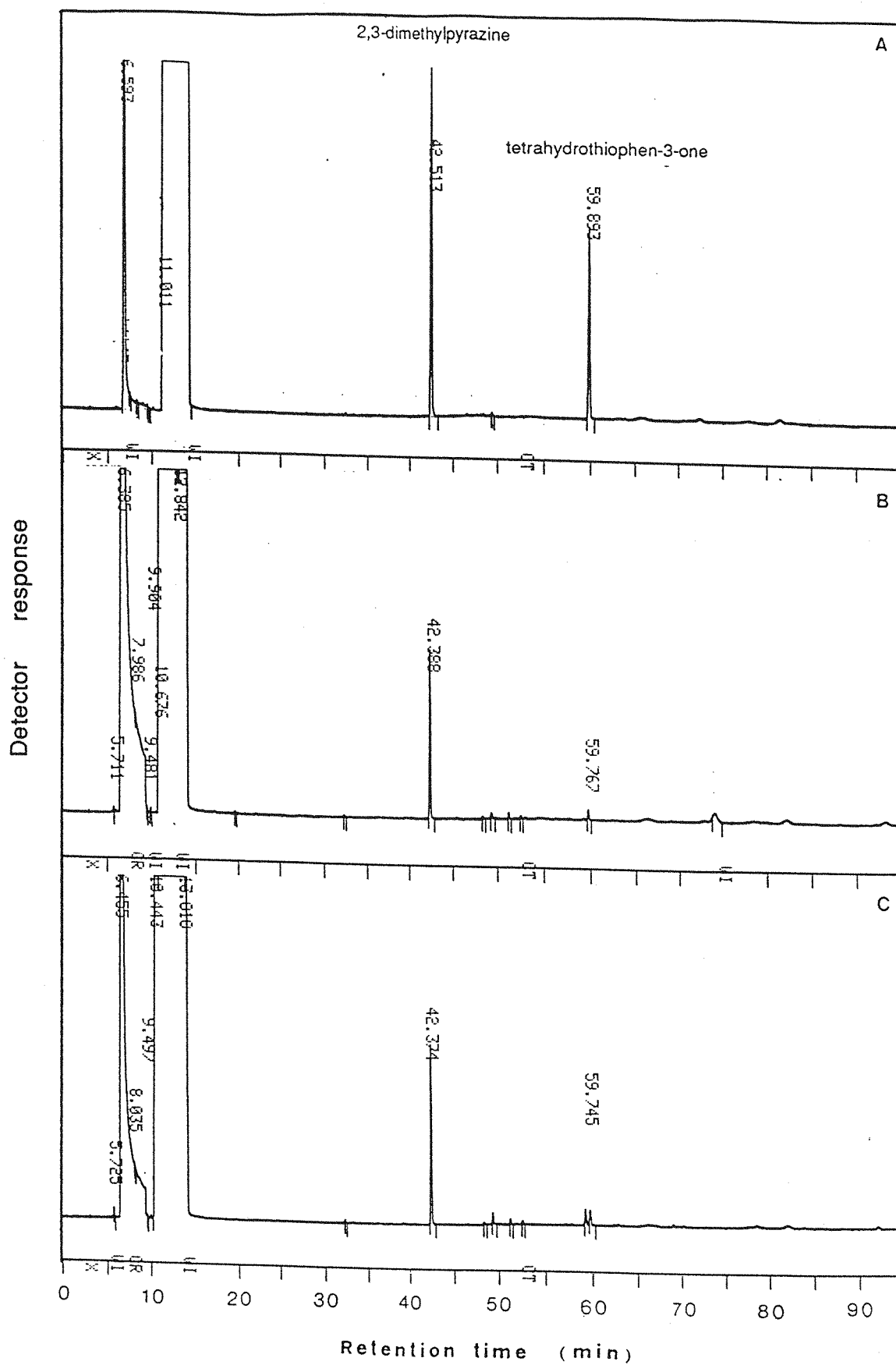


Fig. 14 Gas chromatography of MRP standards (A) and boiled pork juice during various boiling periods : 0 (B), 1 (C), 2 (D), 4 (E), 8 (F), 12 (G), 16 (H), 20 (I) hr.

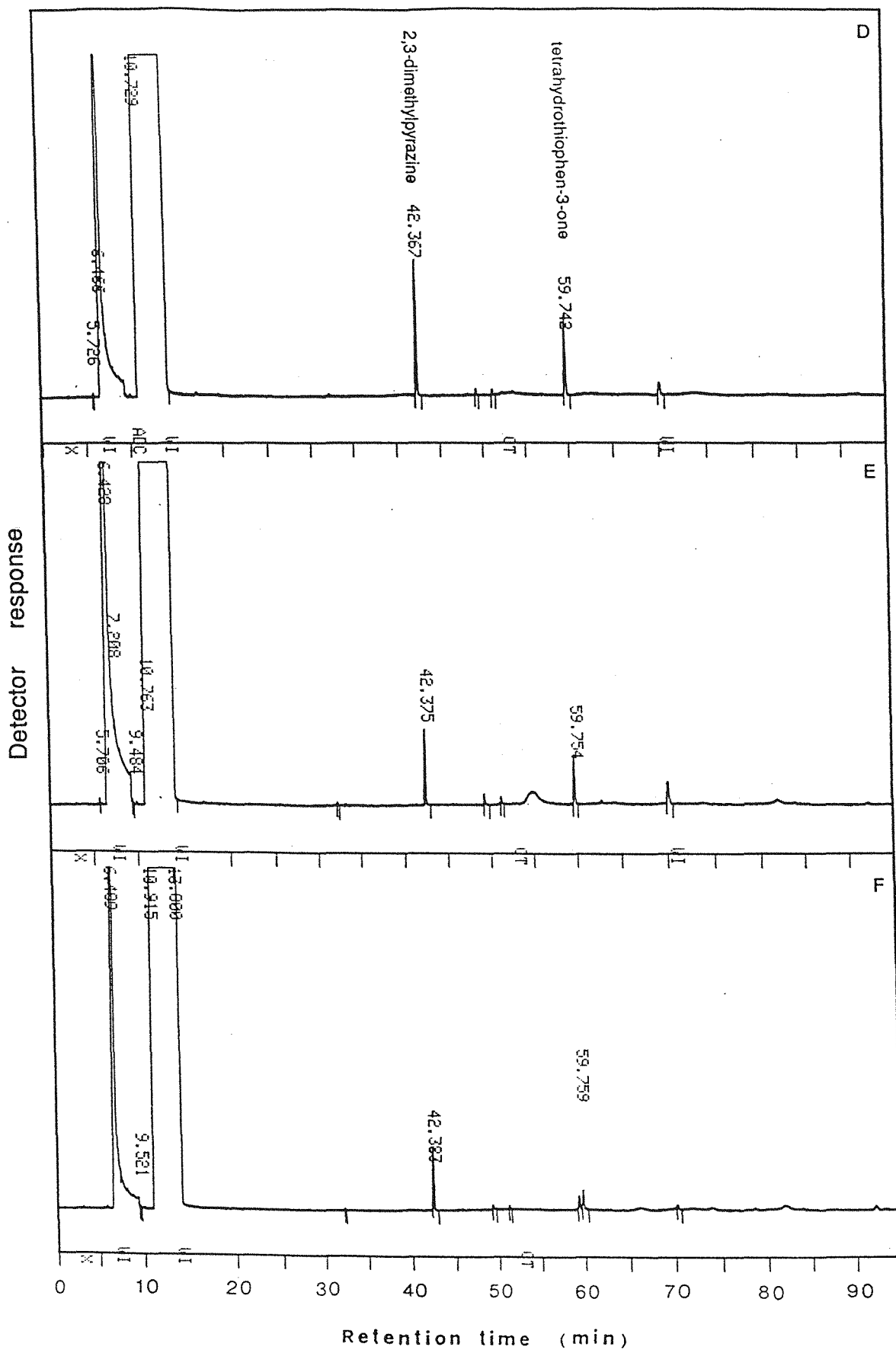


Fig. 14 (continued)

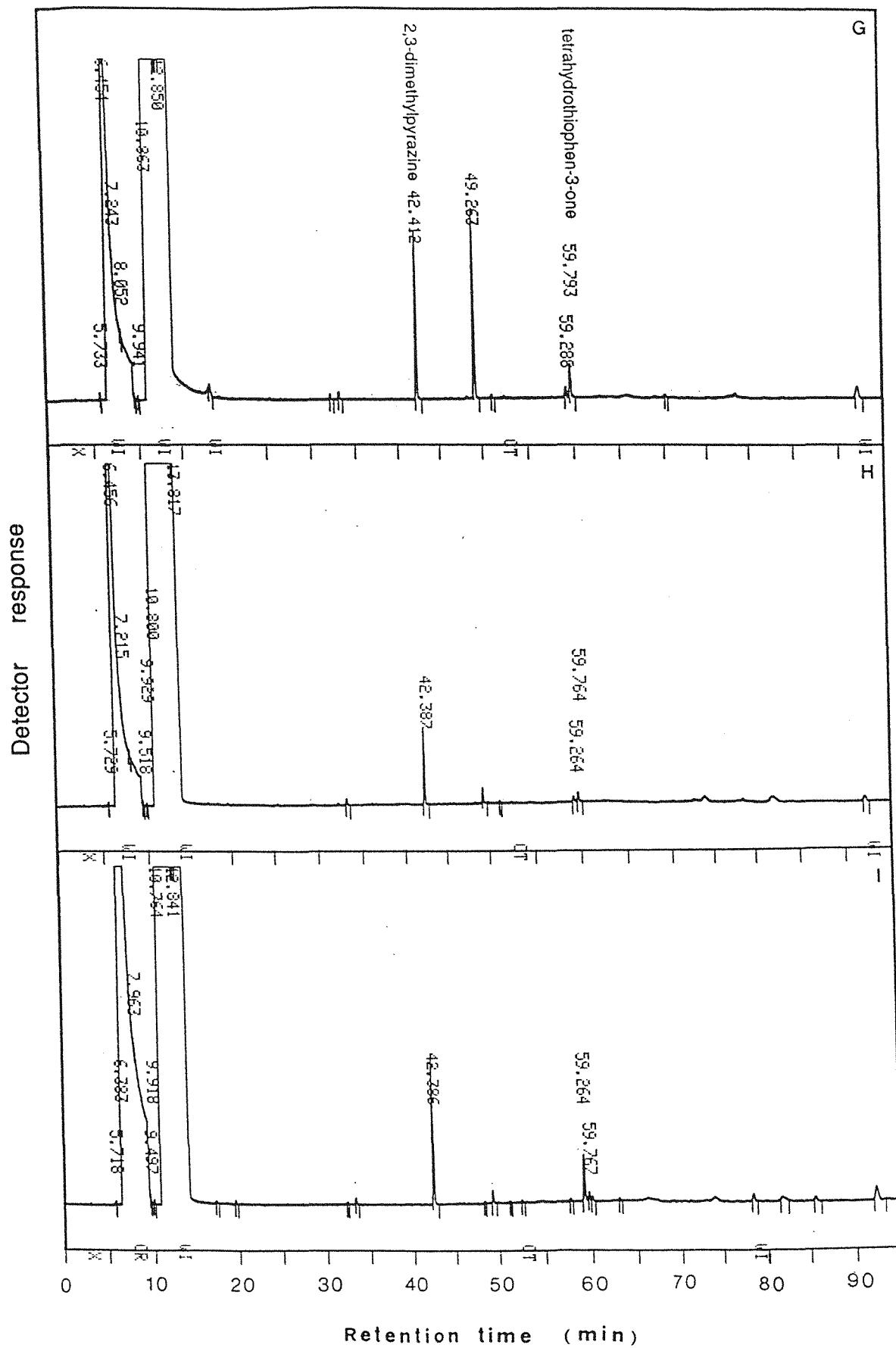


Fig. 14 (continued)

**Table 11** Effect\* of the amount of 2,3-dimethylpyrazine and tetrahydrothiophen-3-one on the mutagenicity of pork extracts during the boiling period. The residue of 2,3-dimethylpyrazine and tetrahydrothiophen-3-one at each boiling time was analyzed by GC.

Boiling time (hour)	Amount (μmol)			Revertants / plate <sup>d</sup>
	DMP <sup>a</sup>	THT <sup>b</sup>	[Ratio] <sup>*</sup> DMP+THT <sup>c</sup>	
0	20.72	2.45	23.17	43 ± 5
1	22.29 [1.08]	4.37 [1.78]	26.66 [1.15]	53 ± 6
2	16.54 [0.80]	19.46 [7.94]	36.00 [1.55]	55 ± 6
4	9.19 [0.44]	12.08 [4.93]	21.27 [0.92]	61 ± 8
8	7.82 [0.38]	5.19 [2.12]	13.01 [0.56]	346 ± 31
12	20.85 [1.01]	7.78 [3.18]	28.63 [1.24]	281 ± 32
16	9.42 [0.45]	2.25 [0.92]	11.67 [0.50]	866 ± 66
20	18.56 [0.90]	2.44 [1.00]	21.00 [0.91]	2419 ± 219

DMP: 2,3-dimethylpyrazine; THT: tetrahydrothiophen-3-one.

The mutagenicity was tested with *Salmonella typhimurium* TA98 in the presence of S9 mix and one percent of total basic extracts was used in the mutagenicity testing per plate.

\* Ratio : the amount of DMP or THT during boiling time / the amount of DMP or THT before boiling.

<sup>a</sup> No significant correlation between a and d:  $r = -0.31, P > 0.05$

<sup>b</sup> Correlation between b and d:  $r = -0.82, P < 0.1$

<sup>c</sup> Correlation between c and d:  $r = -0.71, P < 0.1$



Table 12 Effect\* of the addition of 2,3-dimethylpyrazine on the mutagenicity of pork extracts during the boiling period. The residue of 2,3-dimethylpyrazine at each boiling time was analyzed by GC.

Boiling time (hour)	Residue of DMP (mmol)	No. of revertants / plate
0	4.962	40 ± 5
1	4.684	36 ± 4
2	4.495	43 ± 3
4	3.852	47 ± 4
8	3.373	280 ± 26
12	2.128	488 ± 38
16	2.089	338 ± 27
20	1.686	248 ± 34

BPE: boiled pork extracts ; DMP: 2,3-dimethylpyrazine.

5.0 mmol 2,3-dimethylpyrazine was added to boiled pork model systems at pH 6.0 condition.

The mutagenicity was tested with *Salmonella typhimurium* TA98 in the presence of S9 mix and one percent of total basic extracts was used in the mutagenicity testing per plate.

\* Significant correlation :  $r = -0.85, P < 0.01$

**Table 13** Effect\* of the addition of tetrahydrothiophen-3-one on the mutagenicity of pork extracts during the boiling period and the residue of tetrahydrothiophen-3-one at each boiling time was analyzed by GC.

Boiling time (hour)	Residue of THT (mmol)	No. of revertants / plate
0	4.809	44 ± 6
1	3.912	418 ± 35
2	2.123	1077 ± 129
4	1.376	413 ± 32
8	0.391	1171 ± 90
12	0.107	1925 ± 221
16	0.056	1537 ± 222
20	0.030	2381 ± 322

THT: tetrahydrothiophen-3-one

5.0 mmol tetrahydrothiophen-3-one was added to boiled pork model systems at pH 5.0 condition.

The mutagenicity was tested with *Salmonella typhimurium* TA98 in the presence of S9 mix and one percent of total basic extracts was used in the mutagenicity testing per plate.

\* Significant correlation :  $r = -0.83, P < 0.02$

**Table 14** Effects of the addition of Maillard reaction products to boiled pork extracts model system on the mutagenicity of basic extract with *S. typhimurium* TA98 in the presence of S9 mix.

The addition of Maillard reaction product	No. of revertants / plate ( ratio to BPE )
BPE	704 ± 109
BPE + tetrahydrothiophen-3-one	2041 ± 232 (2.90)
BPE + 2,3-dimethylpyrazine	1152 ± 95 (1.64)
BPE + 3-methylpyridine	1076 ± 99 (1.52)
BPE + 2-methylthiophene	866 ± 58 (1.23)
BPE + 2-acetylpyrrole	236 ± 16 (0.34)
BPE + imidazole	142 ± 34 (0.20)

BPE: boiled pork extracts.

5.0 mmol Maillard reaction product was added to boiled pork model systems at pH 6.0 condition.

The mutagenicity was tested with *Salmonella typhimurium* TA98 in the presence of S9 mix and 2 percent of total basic extracts were used in the mutagenicity testing per plate.

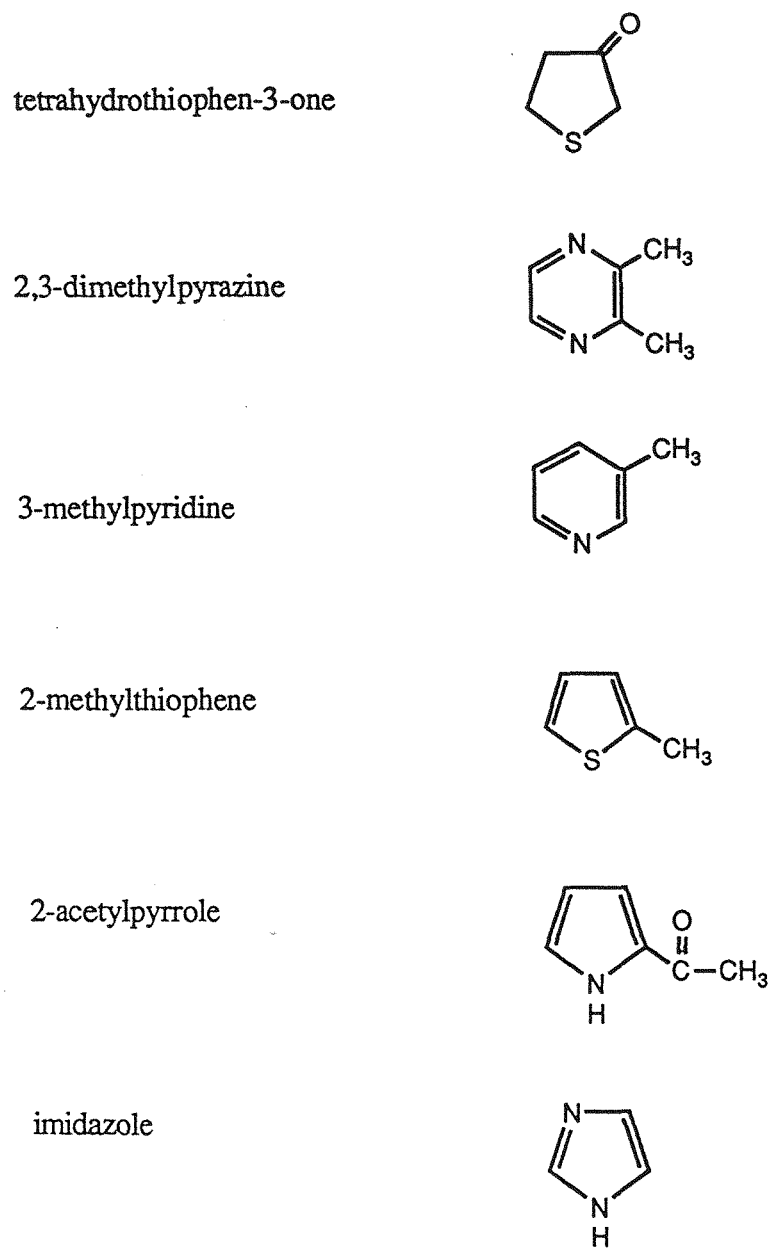


Fig. 15 The structure of some Maillard reaction products.

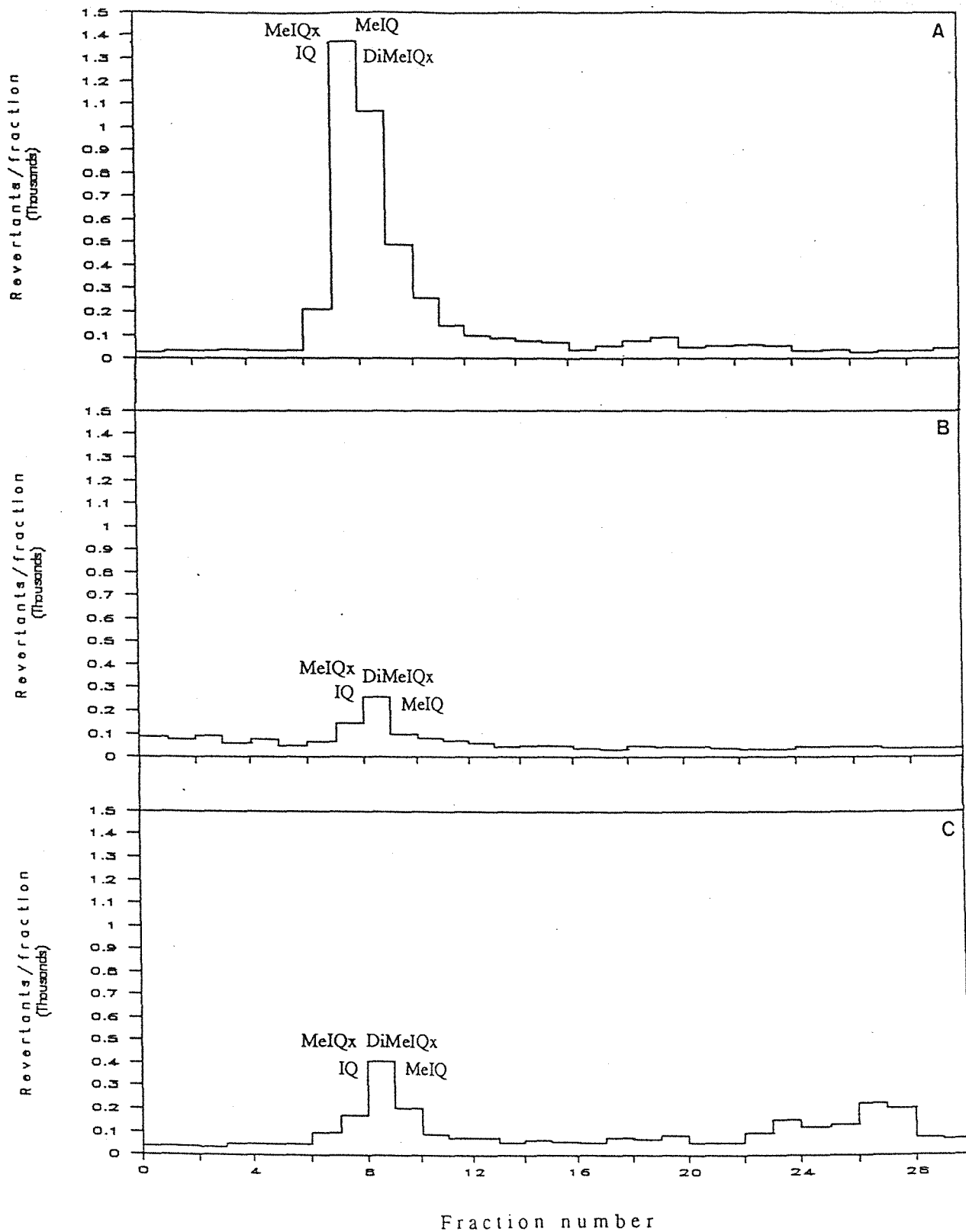


Fig. 16 The mutagenic profiles of semipreparative HPLC fractionation of boiled pork extracts with or without (A) the addition of imidazole (B), 2-acetylpyrrole (C).

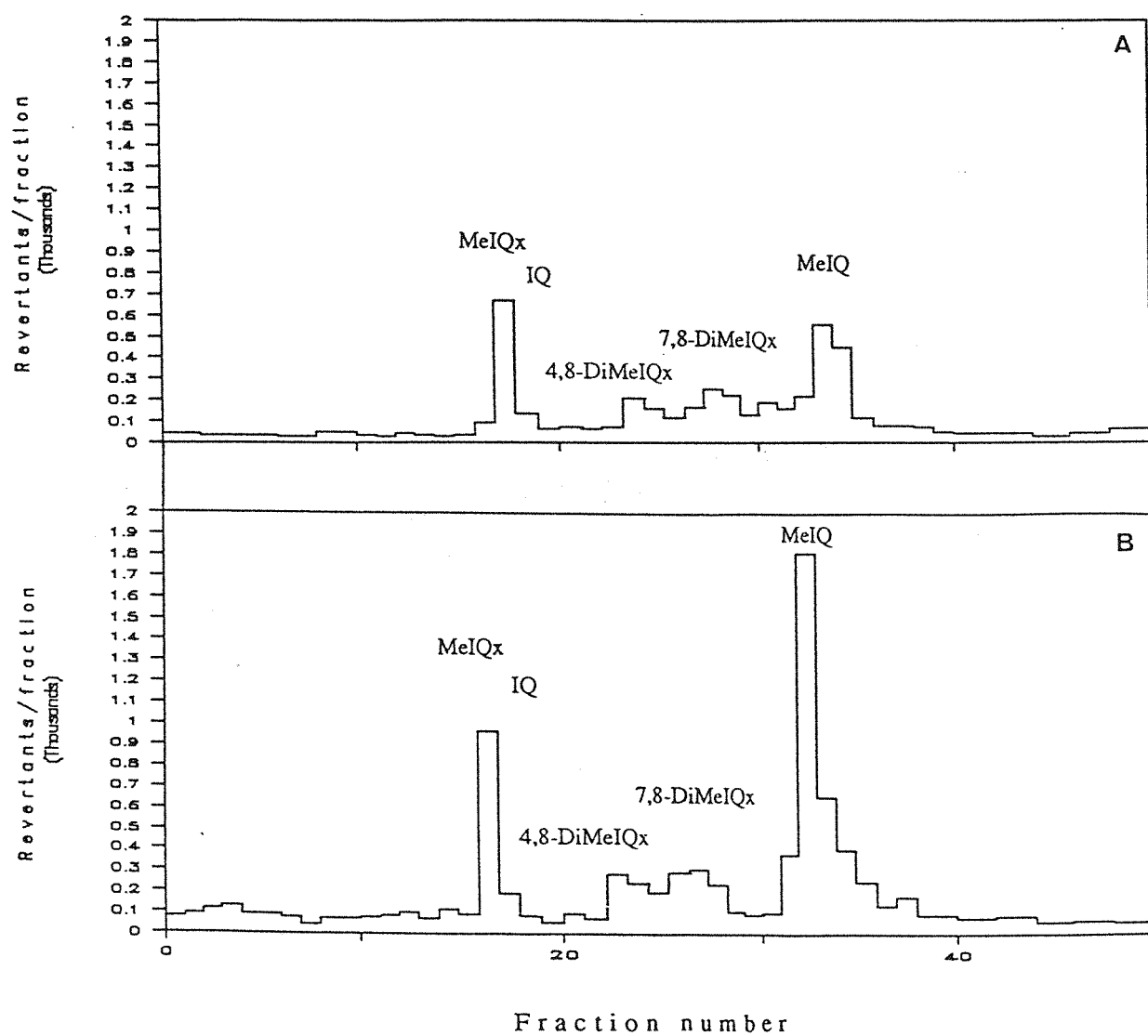


Fig. 17 The mutagenic profiles of HPLC fractionation of boiled pork extracts with or without(C) the addition of 2,3-dimethylpyrazine(A), tetrahydrothiophen-3-one(B), imidazole(D), 2-acetylpyrrole(E).

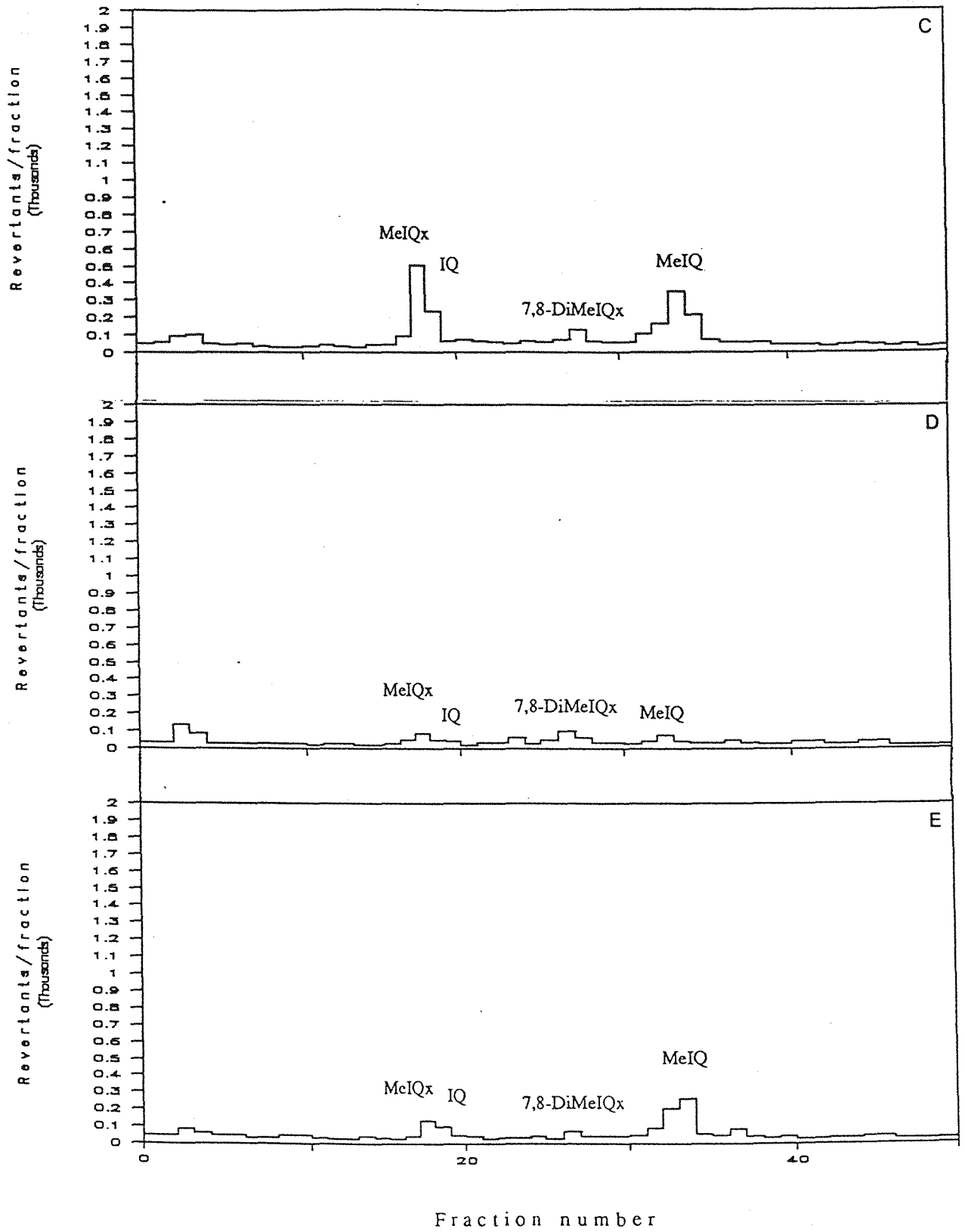


Fig. 17 (continued)

**Table 15** Effects of the addition of Maillard reaction product on the mutagen formation of boiled pork extracts by HPLC fractionation. The mutagenicity of HPLC elutions was tested with *S. typhimurium* TA98 in the presence of S9 mix.

Model	Net revertants / fraction (ratio to BPE)				
	Unknown	MeIQx, IQ	4,8-DiMeIQx	7,8-DiMeIQx	MeIQ
BPE	75	677	ND	86	606
BPE+THT	97(1.29)	981 (1.45)	375	886 (10.30)	3206 (5.29)
BPE+DMP	ND	715 (1.06)	310	521 (6.06)	1230 (2.03)
BPE+AP	27(0.36)	115 (0.17)	ND	10 (0.12)	288 (0.48)
BPE+Imidazole	102(1.36)	27 (0.04)	ND	45 (0.52)	14 (0.02)

M.R.P. : THT: tetrahydrothiophen-3-one; DMP: 2,3-dimethylpyrazine; AP: 2-acetylpyrrole.

5.0 mmol Maillard reaction product was added to boiled pork model systems.

ND: not detected.

The mutagenicity was tested with *Salmonella typhimurium* TA98 in the presence of S9 mix and 13 percent of total basic extracts were used in the mutagenicity testing.



**Table 16 Mutagenicity and browning intensity of boiled pork extracts with or without Maillard reaction product at various pH conditions.**

pH value	BPE <sup>a</sup>		BPE+DMP <sup>b</sup>		BPE+THT <sup>c</sup>	
	Revertants /plate	Browning intensity	Revertants /plate	Browning intensity	Revertants /plate	Browning intensity
3.0	112 ± 9	3.704	188 ± 18	3.272	668 ± 50	3.641
5.0	393 ± 35	1.336	140 ± 7	0.836	1569 ± 119	0.792
6.0	192 ± 18	0.408	335 ± 10	0.680	1019 ± 105	1.121
7.0	153 ± 14	0.439	286 ± 14	0.399	1144 ± 108	0.730
9.0	489 ± 50	0.376	298 ± 19	0.361	1397 ± 130	2.215
11.0	450 ± 42	0.296	268 ± 12	0.283	1284 ± 64	2.214

<sup>a</sup> BPE: boiled pork extracts.

<sup>b</sup> BPE + DMP: boiled pork extracts model system with 2,3-dimethylpyrazine.

<sup>c</sup> BPE + THT : boiled pork extracts model system with tetrahydrothiophen-3-one.

5.0 mmol Maillard reaction product was added to boiled pork model systems.

Browning intensity : absorbance at 420 nm. ( 10-fold dilution with distilled water )

The mutagenicity was tested with *Salmonella typhimurium* TA98 in the presence of S9 mix and one percent of total basic extracts was used in the mutagenicity testing per plate.

**Table 17 Mutagenicity and browning intensity of boiled pork extracts with and without 2,3-dimethylpyrazine or tetrahydrothiophen-3-one during various boiling times.**

Time from boiled beginning (hour)	BPE <sup>a</sup>		BPE+DMP <sup>b</sup>		BPE+THT <sup>c</sup>	
	Revertants / plate	Browning intensity	Revertants / plate	Browning intensity	Revertants / plate	Browning intensity
1	53±6	0.152	36±4	0.157	418±35	0.243
2	55±6	0.191	43±3	0.187	1077±129	0.342
4	61±8	0.380	47±4	0.293	413±32	0.346
8	346±31	0.578	280±26	0.490	1171±90	0.734
12	281±32	0.625	488±38	0.440	1925±221	0.686
16	866±66	0.698	338±27	0.455	1537±222	0.474
20	2419±219	0.926	248±34	0.466	2381±322	0.912

BPE: boiled pork extracts.

5.0 mmol Maillard reaction product was added to boiled pork model systems.

Browning intensity : absorbance at 420 nm. ( 10-fold dilution with distilled water )

<sup>a</sup> Significant correlation :  $r=0.82, P < 0.05$

<sup>b</sup> Significant correlation :  $r=0.83, P < 0.05$

<sup>c</sup> Significant correlation :  $r=0.85, P < 0.02$

**Table 18 Mutagenicity and browning intensity of boiled pork extracts with 2,3-dimethylpyrazine or tetrahydrothiophen-3-one at various concentrations.**

Added concentration of Maillard reaction product (mmol)	BPE+DMP <sup>a</sup>		BPE+THT <sup>b</sup>	
	Revertants /plate	Browning intensity	Revertants /plate	Browning intensity
0	309 ± 17	0.547	294 ± 30	0.547
2.5	486 ± 51	0.541	2737 ± 256	0.942
5.0	425 ± 42	0.586	1404 ± 162	0.686
10.0	335 ± 21	0.547	716 ± 40	0.702
20.0	420 ± 39	0.566	523 ± 36	0.706

<sup>a</sup> BPE + DMP: boiled pork extracts model system with 2,3-dimethylpyrazine.

<sup>b</sup> BPE + THT: boiled pork extracts model system with tetrahydrothiophen-3-one.

The pH value in <sup>a</sup> BPE+DMP model was 6.0.

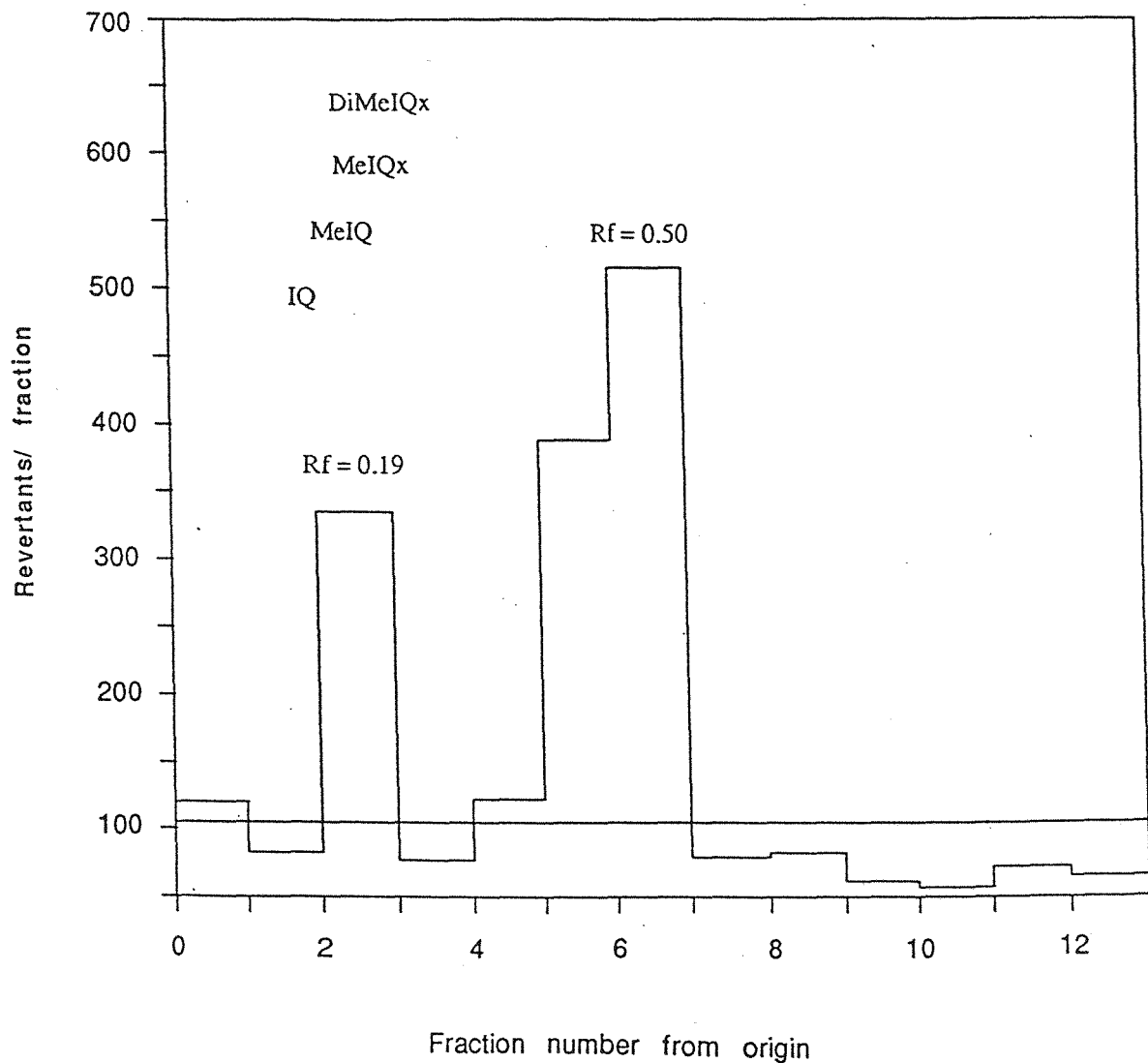
The pH value in <sup>b</sup> BPE+THT model was 5.0.

Browning intensity : absorbance at 420 nm. ( 10-fold dilution with distilled water )

The mutagenicity was tested with *Salmonella typhimurium* TA98 in the presence of S9 mix and one percent of total basic extracts was used in the mutagenicity testing per plate.

<sup>a</sup> No significant correlation :  $r= 0.21, P > 0.05$

<sup>b</sup> Significant correlation :  $r= 0.91, P < 0.05$



**Fig. 18** The profile of TLC fractionations from extracts of 2-methylpyridine, acetylformaldehyde and creatinine model system. 2 x Background revertant level was indicated with a line.

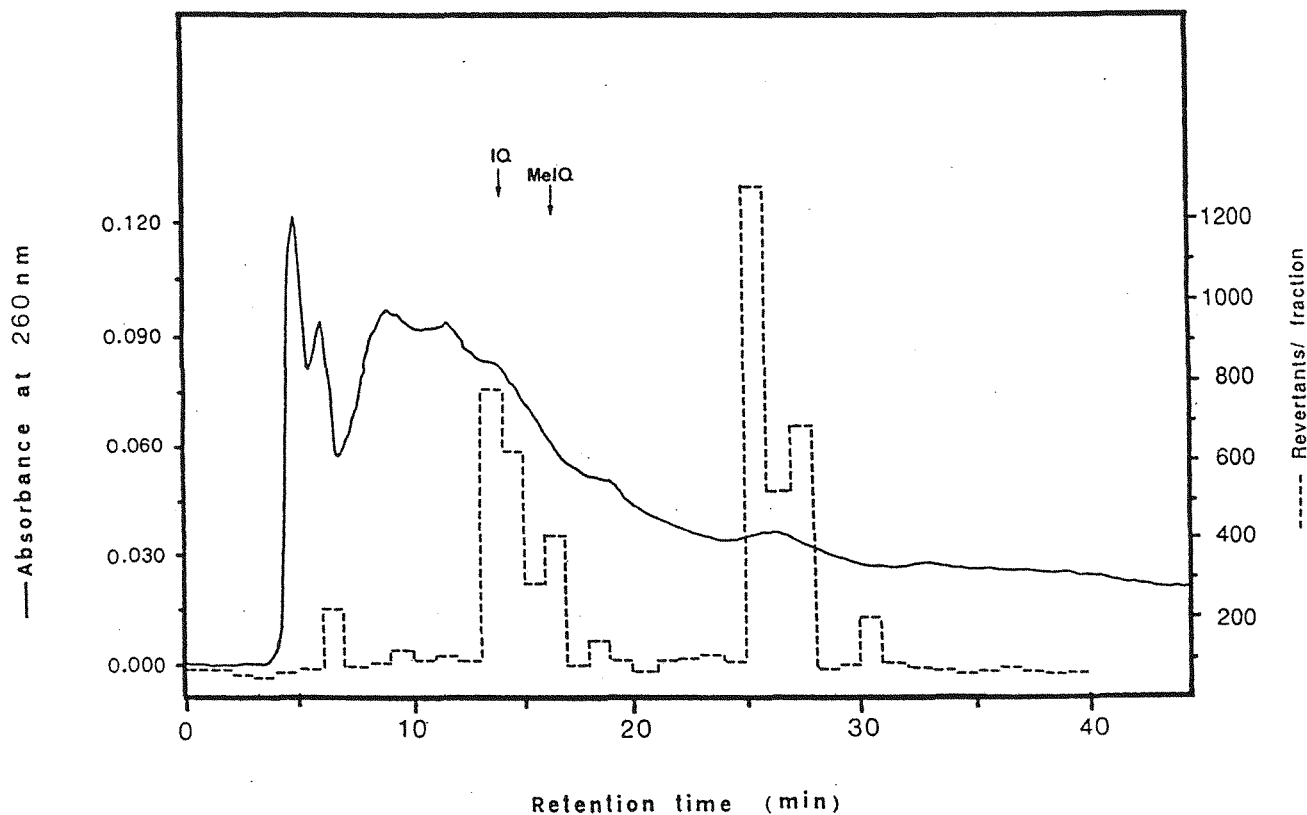


Fig. 19 The elution profile of the extracts of 2-methylpyridine, acetylformaldehyde and creatinine heated model system by semipreparative HPLC on an ODS column. The mutagenicity of 1-min fraction was tested with *S. typhimurium* TA98 in the presence S9 mix.

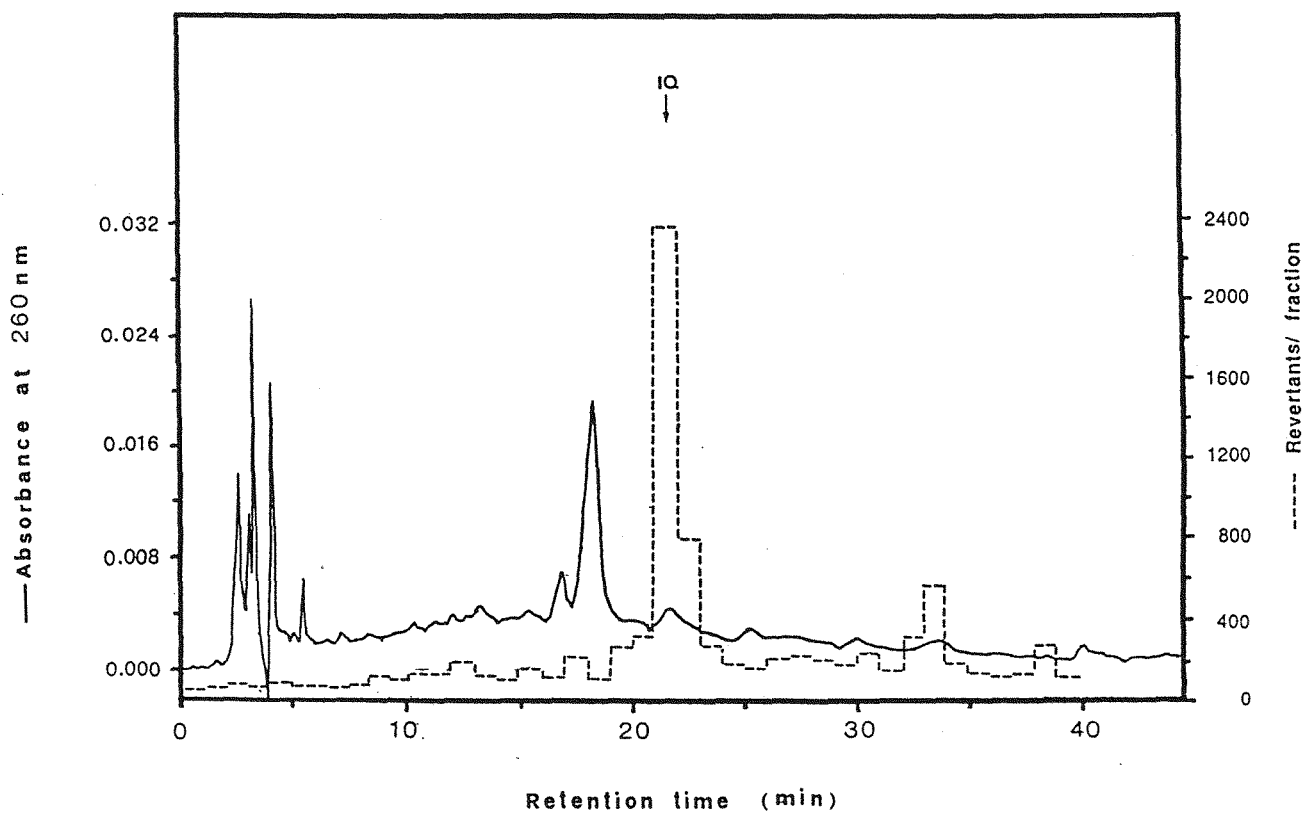
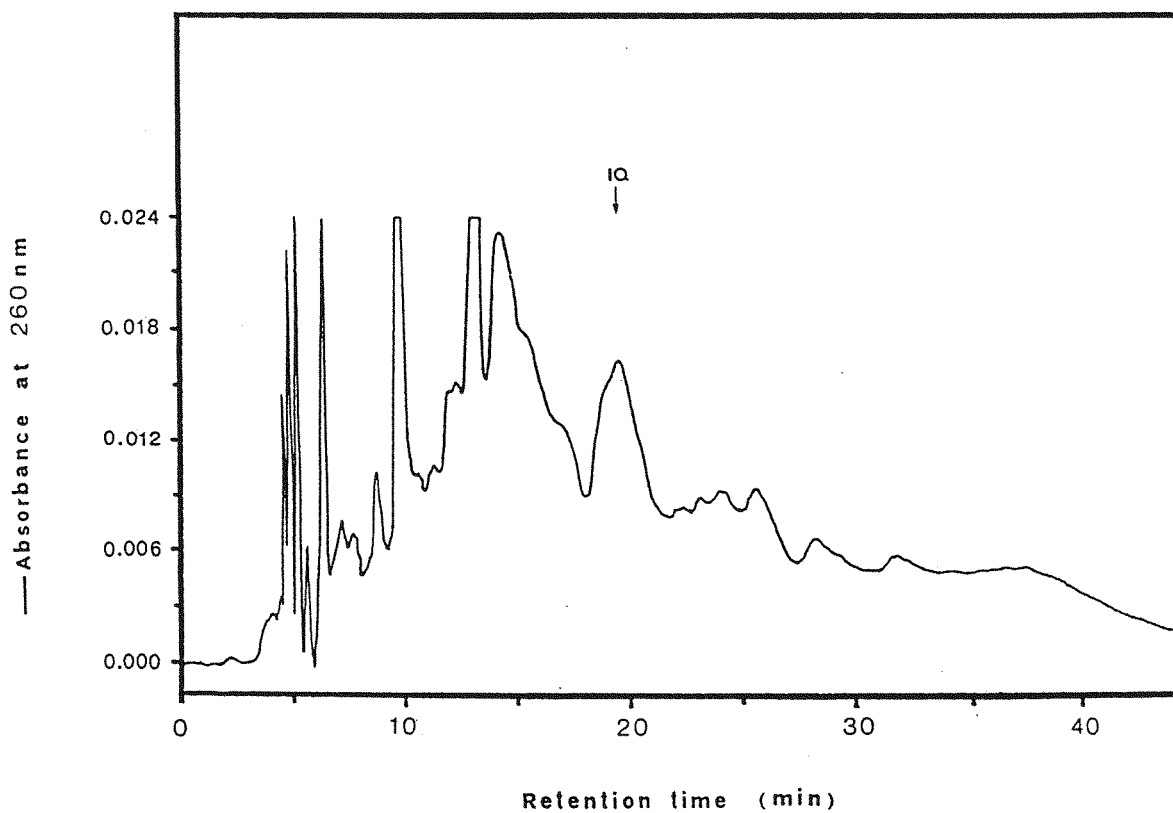
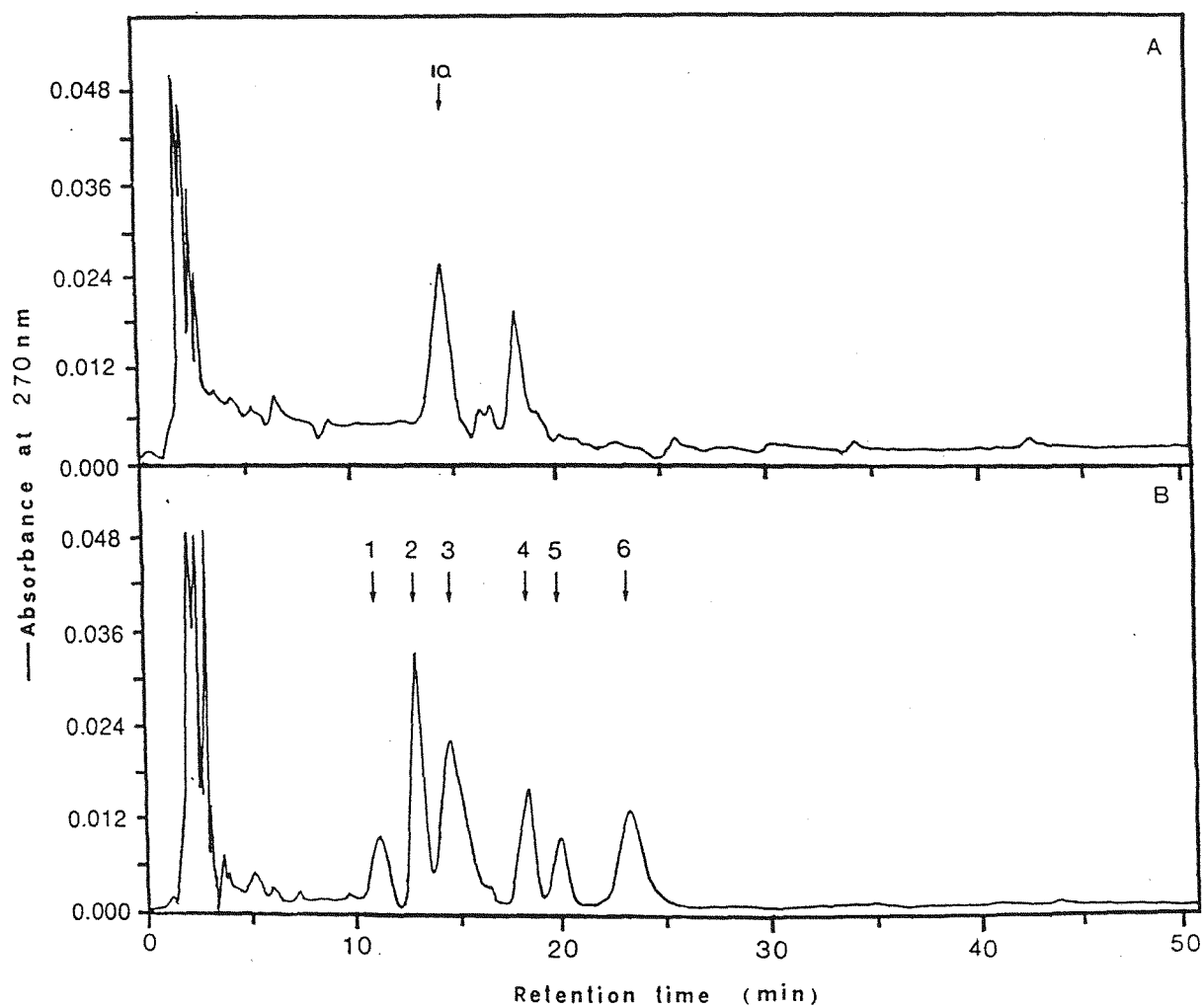


Fig. 20 The elution profile of the extracts of 2-methylpyridine, acetylformaldehyde and creatinine heated model system by HPLC on an analytical Nucleosil 5 CN column. The mutagenicity of 1-min fraction was tested using *S. typhimurium* TA98 with S9 mix.

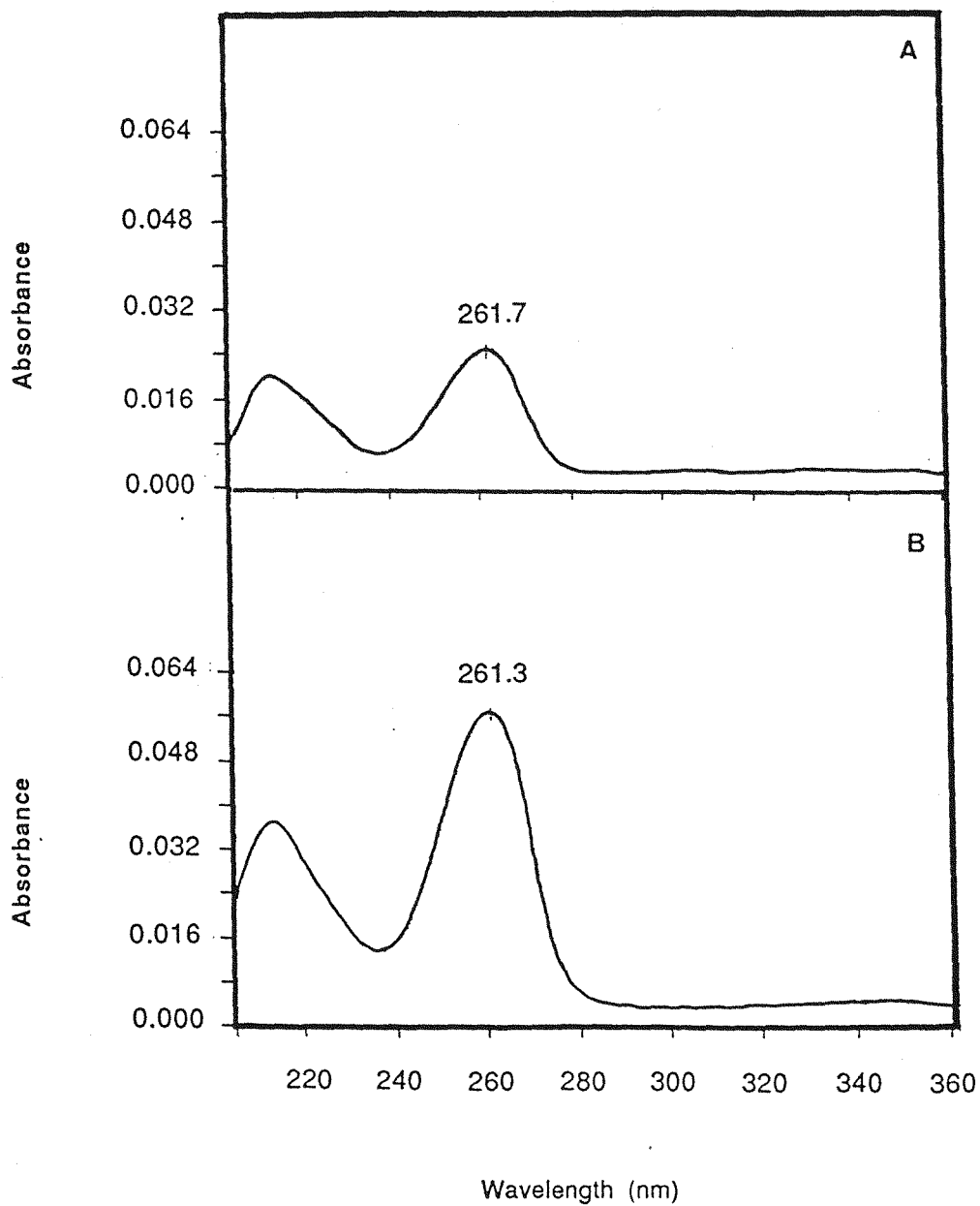


**Fig. 21** The elution profile of the mutagens in 2-methylpyridine, acetyl-formaldehyde and creatinine heated model system by 2nd analytical HPLC on a Nucleosil 5 CN column.



**Fig. 22** Elution profiles of the mutagens in 2-methylpyridine, acetylformaldehyde and creatinine heated model system (A) and authentic heterocyclic amines (B) by HPLC on an analytical Spherisorb pH Stable 5 C<sub>18</sub> column. 1= DMIP; 2=MeIQx; 3=IQ; 4=7,8-DiMeIQx; 5=4,8-DiMeIQx; 6=MeIQ.





**Fig. 23** Ultraviolet absorbance spectra of purified fraction of 2-methylpyridine, acetylformaldehyde and creatinine heated model system (A) and authentic IQ (B) obtained from photodiode array detector by HPLC.

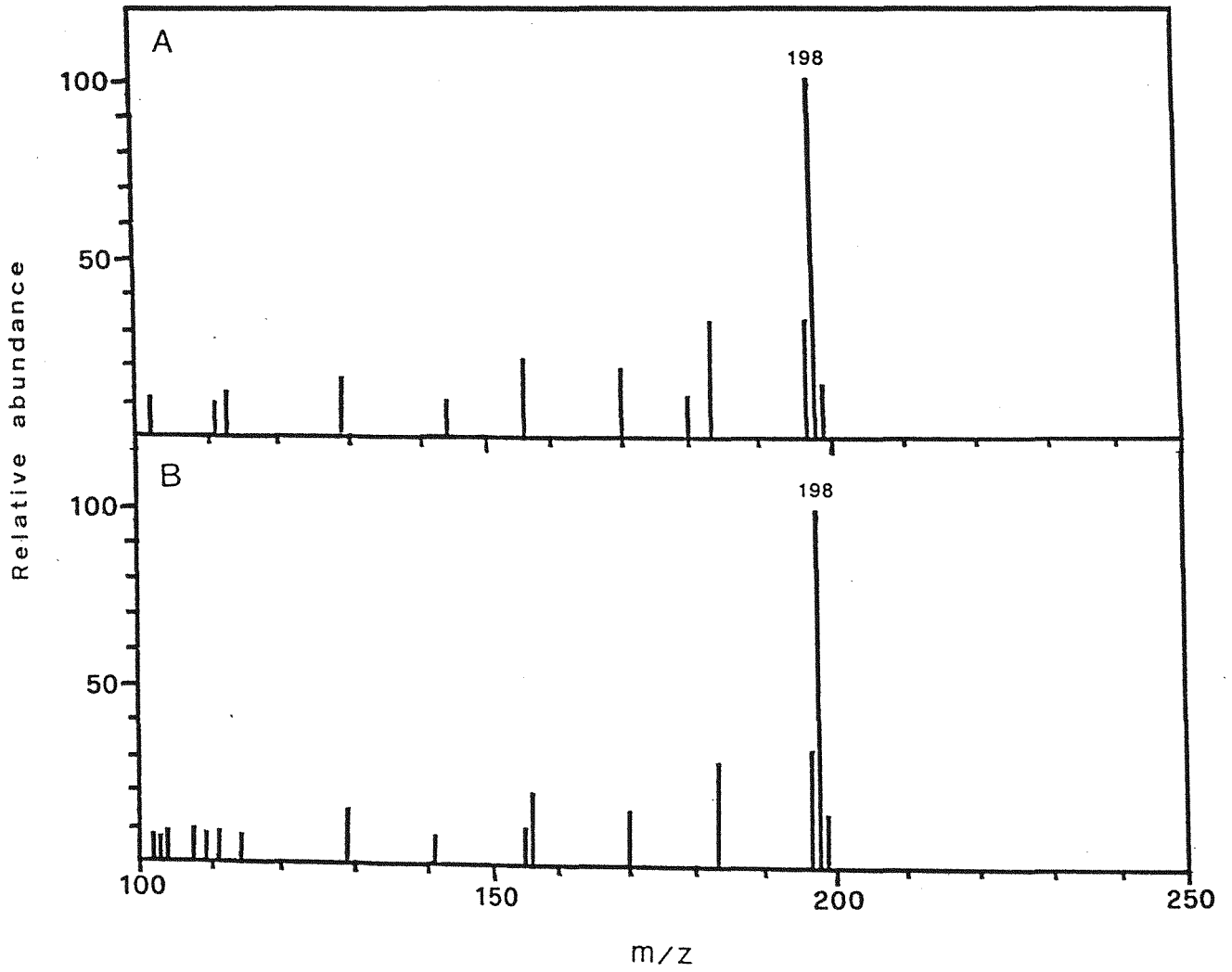


Fig. 24 Mass spectra of authentic IQ (A) and IQ isolated from 2-methylpyridine, acetylformaldehyde and creatinine heated for 1 h at ca. 150°C (B). Fragments occurred at less than m/z 100 were not shown.

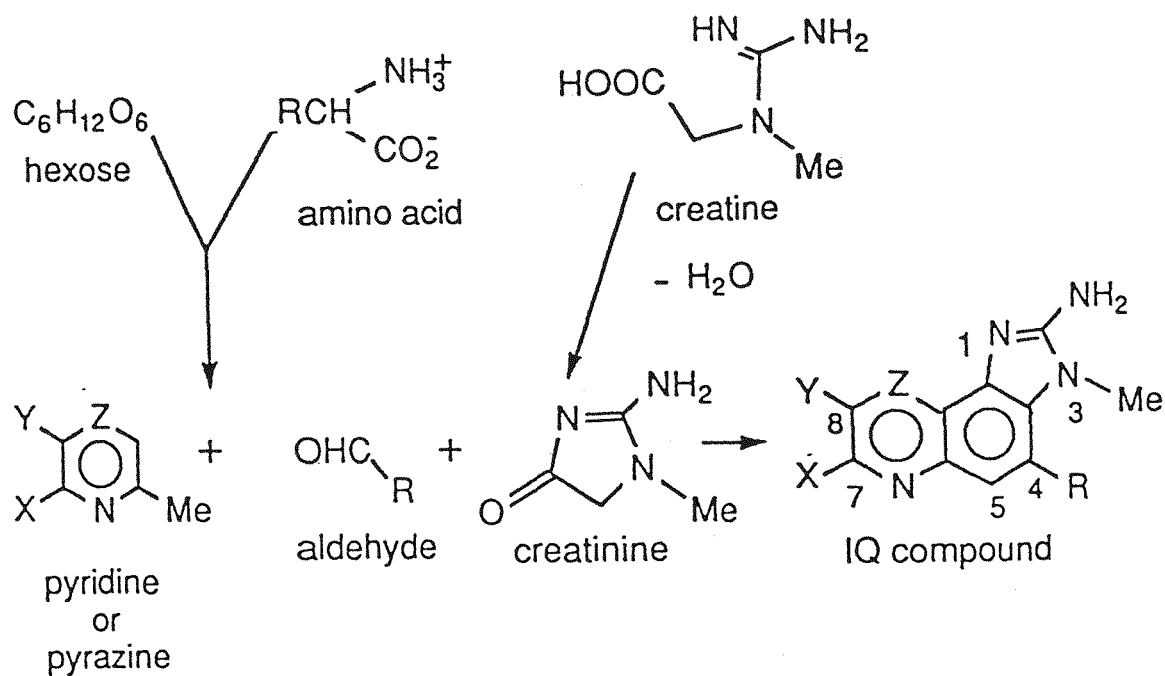
**Table 19 Comparison of Maillard reaction product and creatinine with or without aldehyde model systems on the mutagenicity of basic extract with *S. typhimurium* TA98 in the presence of S9 mix.**

Model	No. of revertants / plate *
Pyrazine + acetaldehyde + creatinine	930±74
2-Methylpyridine + acetaldehyde + creatinine	230±29
2-Methylpyridine + acetylformaldehyde + creatinine	922±46
2-Methylpyridine + diacetyl + creatinine	1448±154
Tetrahydrothiophen-3-one + acetylformaldehyde + creatinine	491±50
Tetrahydrothiophen-3-one + creatinine	262±25

The mutagenicity was tested with *Salmonella typhimurium* TA98 in the presence of S9 mix and 1/450th of total basic extracts were used in the mutagenicity testing per plate.

Samples were refluxed for 1 h at ca.150°C in diethylene glycol containing 5% water (DEG -H<sub>2</sub>O) and basic extracts were obtained by XAD-2 fractionation and acid / base partitioning with dichloromethane .

A



B

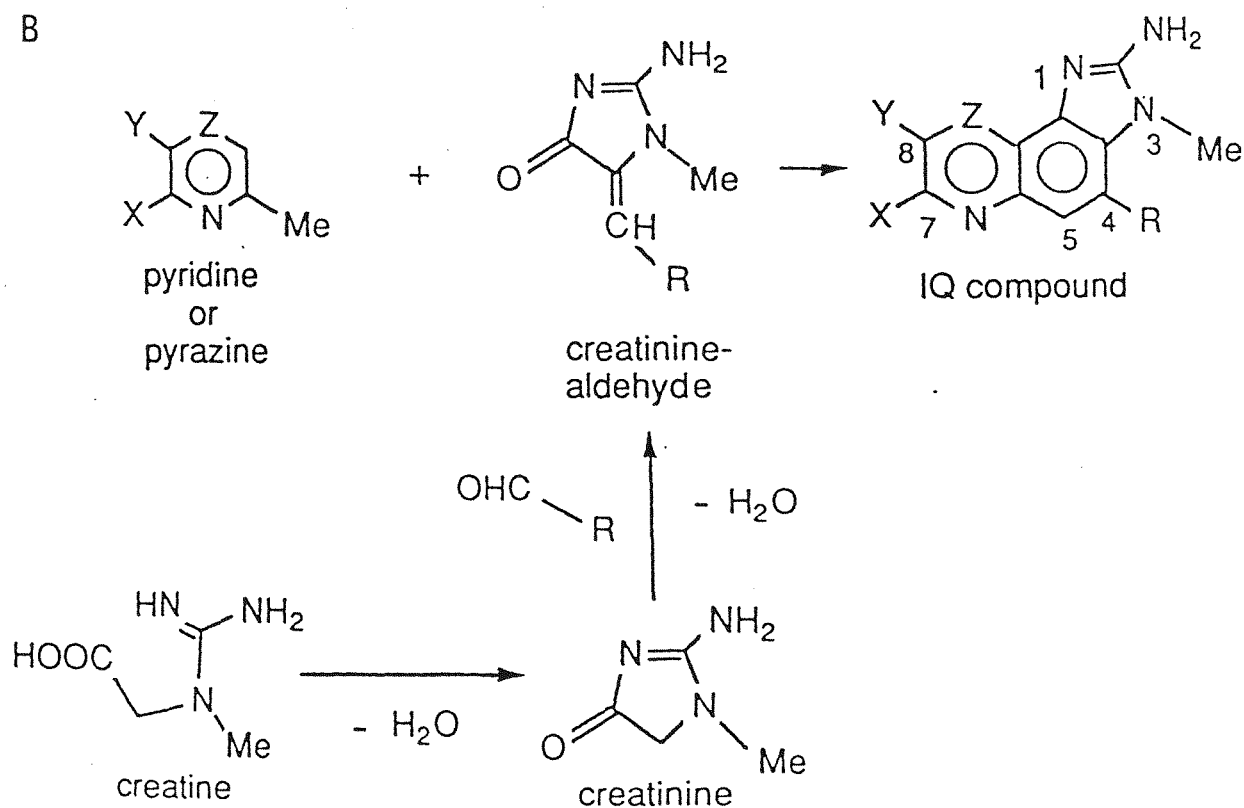
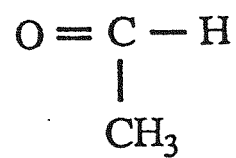
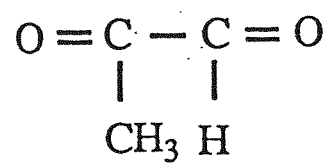


Fig. 25 Postulated reaction route (A) (Jägerstad et al., 1983a) and alternative route (B) (Nyhammar, 1986) for the formation of IQ compounds. R, X, and Y may be H or Me; Z may be CH or N.

acetaldehyde



acetylformaldehyde



diacetyl

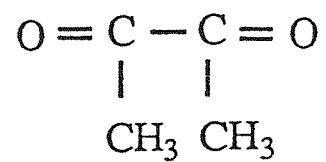


Fig. 26 The structure of some aldehydes.

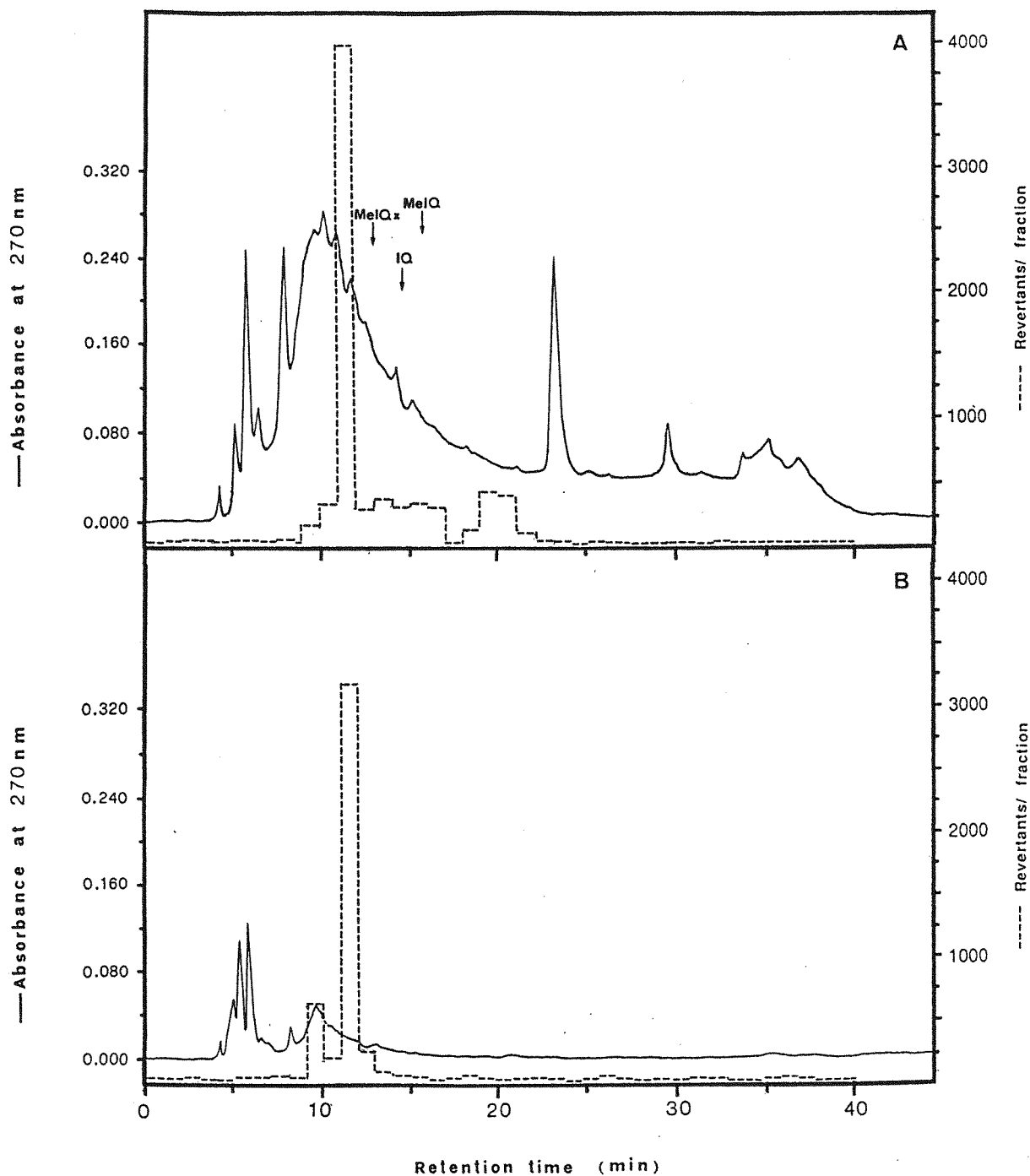


Fig. 27 Elution profiles of the extracts of tetrahydrothiophen-3-one and creatinine with (A) or without (B) acetylformaldehyde heated model systems by semipreparative HPLC on an Nucleosil column. The mutagenicity of 1-min fraction was tested using *S. typhimurium* TA98 with S9 mix.

## 捌。英文摘要

The boiled pork juice was refluxed at 102°C for 12 hr and then basic extracts of the mixture was found to be mutagenic toward *Salmonella typhimurium* TA98 with S9 mix. The mutagenic fractions were purified by blue cotton extraction and high performance liquid chromatography (HPLC). The HPLC fractionation followed by mutagenicity testing yielded mutagenicity profiles with peaks in areas where the heterocyclic amines standards appeared. The mutagenic fractions were collected and confirmed by the comparison of UV absorption spectra, strain specificity, and acid nitrite treatment. Finally, mutagens were identified as MeIQx, IQ and MeIQ by mass spectrometry (MS). One gram of boiled pork juice was estimated to contain 4.1 ng of MeIQx, 3.7 ng of IQ, and 1.2 ng of MeIQ, which accounted for 19.0%, 27.3% and 34.3%, respectively, of the total mutagenicity. The remaining mutagenic fraction might be DiMeIQx isomer which will be further confirmed by MS.

The possible mutagenic precursors in boiled pork extracts, such as glucose, ribose, alanine, creatinine, and some Maillard reaction products. They were added to boiled pork juice model system separately to examine the effect of these compounds on the mutagen formation in boiled pork extracts. The results showed that the addition of glucose, alanine, creatinine, and ribose to the boiled pork juice enhanced 2.7-6.7 folds mutagenicity of boiled pork extracts. The four Maillard reaction products, 2-methylthiophene, 3-methylpyridine, 2,3-dimethylpyrazine, and tetrahydrothiophen-3-one, also increased the formation of mutagens about 1.2-2.9 folds. However, imidazole and 2-acetylpyrrole significantly inhibited the mutagenicity of boiled pork extracts (0.2 and 0.3 folds). Thus, we suggested that glucose, ribose, alanine, creatinine, 2-methylthiophene, 3-methylpyridine, 2,3-dimethylpyrazine, and tetrahydrothiophen-3-one could be the possible chemical components which participated the mutagen formation

in the boiled pork juice model system.

Then, two active Maillard reaction products, tetrahydrothiophen-3-one and 2,3-dimethylpyrazine, were added to boiled pork juice and estimated the optimal reaction conditions to enhance the mutagenicity. Data showed that 2.5 mmol tetrahydrothiophen-3-one was added to boiled pork juice boiled for 20 hr at pH 5.0 had the most highly mutagenicity. However, 2.5 mmol 2,3-dimethylpyrazine boiled for 12 hr at pH 6.0 was the optimal condition for 2,3-dimethylpyrazine. The HPLC mutagenic profiles of basic extracts of the addition of Maillard reaction products were similar to that of boiled pork extracts. We also evaluated the correlation of the variation of tetrahydrothiophen-3-one and 2,3-dimethylpyrazine in boiled pork juice models during the boiling period with their mutagenicity. Our data showed that the residue of tetrahydrothiophen-3-one and 2,3-dimethylpyrazine during the boiling period were significantly correlated to their mutagenicity, respectively ( $r=-0.83, p<0.02$ ;  $r=-0.85, p<0.01$ ). These findings suggested that tetrahydrothiophen-3-one and 2,3-dimethylpyrazine could participate the chemical pathway of the mutagens formation in boiled pork juice model systems.

Furthermore, the possible pathway of IQ-type mutagen formation proposed by Jagerstad group was studied using a Maillard reaction product / aldehyde / creatinine model. The preliminary data showed the 2-methylpyridine / acetylformaldehyde / creatinine model can produce IQ mutagen indeed. But the optimal reaction conditions and the role of aldehyde in this model system for mutagens formation will be investigated in future.