食物致突變物及致癌物分析方法之研究

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一、前言：

食物中成份的熱解產物在1977年時已經被Nagao, M. et al.由魚類和肉類的燒焦部分偵測到致突變性(1)，而Matsumoto, T., et al.（1977）亦由胺基酸的熱解產物測定到致突變性的存在(2)。另外，還有許多研究報告也都已指出肉類食物中的胺基酸與蛋白質在經過高溫處理、於適當的反應溫度、pH值及水分存在之下，會產生致突變性物質，由於致突變性與致癌性相互聯想，以致上述這些發現隨即開展食物組成份與人類癌症間關係的研究領域。

為了探尋致突變物與癌症的關係，首先必須能由食物的複雜成份中分離出初步致突變性化合物，並予以進一步純化後，最後再做化學結構、特性的確定分析，在此一系列的分析步驟中，為了確實掌握致突變物的分佈，可利用較快速的微生物檢測法來輔助化學分析法的偵測，此即為安氏致突變性測定（Ames／Salmonella test）(3)，目前此方法已經被廣泛用於食品、空氣及水質的初步篩檢工作。本篇文章即是將十餘年來有關食物致突變物質的分析研究上進展，做一綜合性簡要的探討。

二、溶劑萃取法

Commoner et al.（1978）所提出的致突變性物質的萃取方法是在pH值滴定之後，以酸（如HCl）和（NH_4）$_2SO_4$使蛋白質沈澱，然後再用溶劑萃取致突變性有機物(4)。而Uyeta, M., et al.（1979）則試以七種不同極性的溶劑來比較其萃取致突變性物質的效果（Fig.1.）(5)其結果，非極性溶劑（如n-hexane）在致突變物的萃取率上極低，相反地，如chloroform／methanol之混合高極性溶劑，則得到致突變物的高萃取率。此外，Felton et al.（1981）則實驗認為利用acetone直接來萃取乃更為簡易且有效，其步驟見Fig.2。其方法在以溶劑（acetone）將

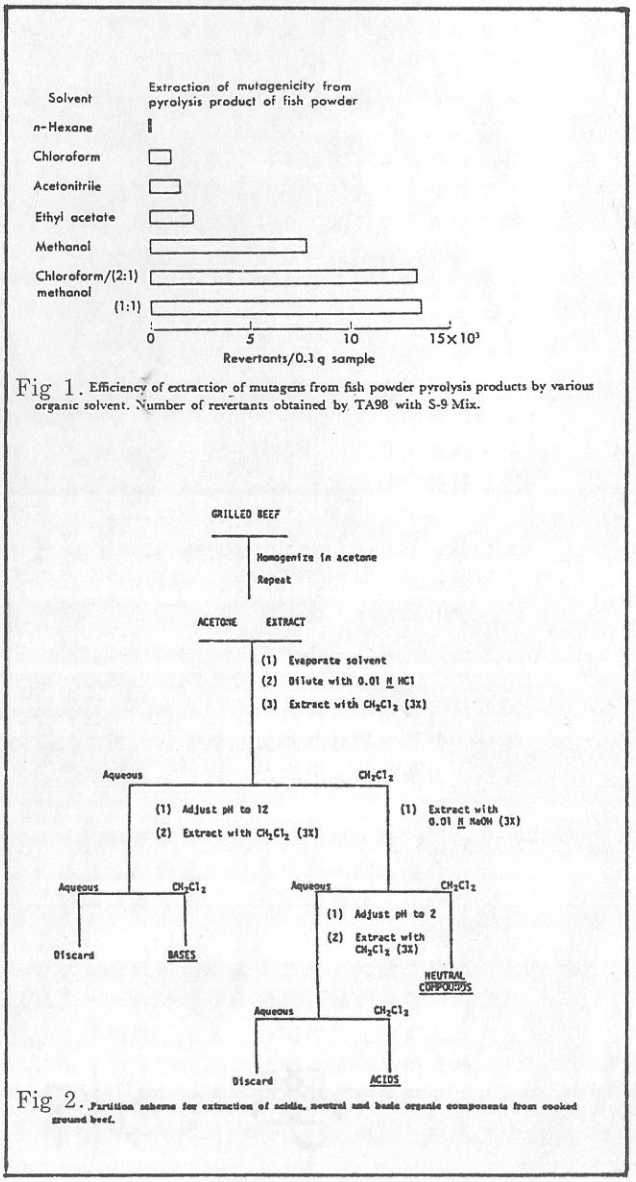


Fig 1. Efficiency of extraction of mutagens from fish powder pyrolysis products by various organic solvent. Number of revertants obtained by TA98 with S-9 Mix.

Fig 2. Partition scheme for extraction of acidic, neutral and basic organic components from cooked ground beef.

初樣品均質化（homogenize）之後予以放冷，如此可引起蛋白質的沈澱，待濾去沈澱後即以酸（HCl）稀釋，接著以CH_2Cl_2萃取出酸性及中性分層，而水層再以鹼（NaOH，NH_4OH）滴定至pH值為12時，以CH_2Cl_2再萃取出鹼性分層，將萃取得之酸性與中性分層分別以五種Salmonella菌屬來檢測致突變性，結果發現都沒有反應（Table 1.）

Table 1.

MUTAGENIC ACTIVITY OF ACID AND NEUTRAL FRACTIONS IN 5 SALMONELLA STRAINS

Fraction	gE/plate [a]	S9 [b]	Strain				
			TA1535	TA1537	TA1538	TA98	TA100
Control	0	—	16 [c]	14	25	21	136
	0	+	32	12	15	17	171
Acid	18	+	13	18	21	27	108
	73	+	9	19	20	28	136
	183	+	8	18	29	27	140
	73	—	17	10	9	18	154
Neutral	1	+	14	12	30	24	165
	3	+	16	20	31	28	150
	9	+	12	13	31	35	183
	27	+	—	—	37	—	—
	3	—	37	10	11	17	158
2-Aminoanthracene	(5 μg)	+	272	436	2262	2623	1014

[a] Equivalent weight in g fresh wt. ground beef (gE).
[b] S9 protein, 2 mg/plate.
[c] Mean number of histidine-revertant colonies per plate (each value is the average from 2 plates).

Table 2.

MUTAGENICITY OF BASIC FRACTIONS EXTRACTED BY ACETONE METHOD

Expt.	Plate	TA1538 revertant colonies [a]						
		Basic fraction of hamburger (gE) [b]						2AA [c] 5 μg
		0	3.2	8.0	16	32	100 [d]	
1	A	17	154	354	777	1285	4688	2344
	B	16	186	378	760	1301		2446
2	A	20	287	513	1117	2162	6890	2389
	B	24	315	819	1132	2117		2329
3	A	16	127	474	860	1140	5299	2495
	B	10	119	421	814	1208		2586
4	A	33	281	654	1170	1981	7138	2127
	B	26	260	649	1155	1917		2647
5	A	21	251	621	1149	1890	7346	3321
	B	30	259	643	1235	2132		3443
Mean							6272	
CV							19%	
SE							532	

[a] Tested in the presence of S9, see Methods for details.
[b] Gram equivalent (gE) of fresh wt. of ground beef (cooked 6 min per side at 200°C).
[c] 2-aminoanthracene.
[d] These data were calculated by least squares regression of the linear portion (32 gE points were usually rejected) of dose—response curves generated from the means of two plates at each dose as seen in Fig. 2.

Table 3.

MUTAGENICITY OF THE BASIC FRACTIONS EXTRACTED FROM COOKED GROUND BEEF WITH ACETONE OR ISOPROPANOL

	Additions	S9 metabolic activation [a]	TA1538 revertant colonies/10^8 cells [b]
Control (no mutagen)			
	—	+	38
	—	—	19
Control (+ mutagen)			
2-Aminoanthracene	5 (μg)	+	1912
3-Methylcholanthrene	50 (μg)	+	143
Extracts			
Acetone	8 gE [c]		373
	16	+	632
	8	—	16
Isopropanol	8	+	368
	16	+	620
	8	—	12

[a] 2 mg protein/plate.
[b] Average of 2 plates.
[c] Equivalent weight in g fresh wt. of ground beef (gE).

(6)。

再取鹼性分層以TA1538來偵測，則有致突變性反應，而且表示此物質乃是造成frame-shift類的突變表現（Table.2.）。另外，還以isopropanol與acetone相比，結果發現此兩溶劑可萃取出等量的致突變活性表現（Table. 3.）。

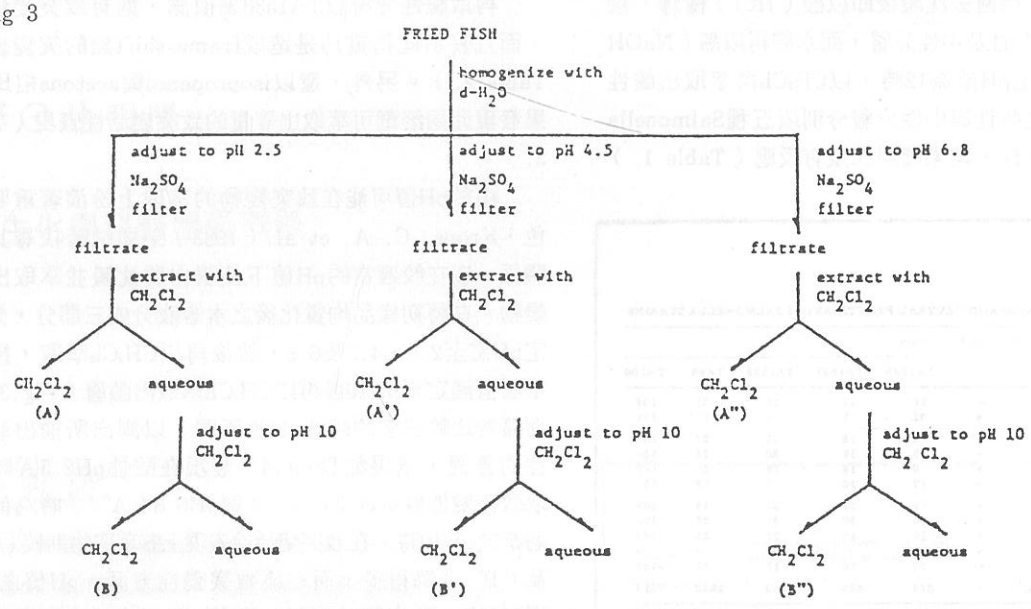
由於pH值可能在致突變物的萃取上扮演著重要的角色，Krone, C. A. et al.（1983）(7)即試著找尋其間的關係，以在較適當的pH值下使蛋白質沈澱並萃取出致突變物。在將初樣品均質化後之水溶液分成三部分，分別滴定pH值至2.5，4.5及6.8，然後再以CH_2Cl_2萃取，接著取水層皆滴定至pH10後再以CH_2Cl_2萃取出鹼層（Fig.3.），而藉著比較三者的起始pH值不同，以測出所抽出致突變性的差異，結果如Table.4，發現在酸性pH2.5(A)時所萃取致突變性較pH4.5（A′）與pH6.8（A″）時為低，不過當於pH10時，在致突變活性表現上(B)萃取物則較（B′）及（B″）高得多，而在總致突變性方面，pH值由6.8低至2.5時，致突變性則增加了約5倍，其可能的解釋為在pH4.5與6.8時，除了致突變物之外的物質可能抑制Ames test的致突變性或在低pH值時可能形成致突變物而在較高pH值時抽出或是致突變物與沈澱蛋白質結合而在pH值增加時將會溶出。

而經以Fig.4來試驗pH值變化對致突變物萃取之影響，可知不論起始的pH值如何，再以pH6.8與10來萃取時，則反應皆很低（樣品Ⅰ′，Ⅱ′，Ⅲ′，Ⅳ′，Ⅴ′，Ⅵ′），因此可知在極酸性的情況下，也不會引起致突變物之生成，反而在pH2.5與10時，其總致突變性則較高（如樣品Ⅰ＋Ⅱ，Ⅲ＋Ⅳ，Ⅴ＋Ⅵ）。由以上結果可知此致突變物是屬於鹼性化合物，且可能與沈澱蛋白質結合，可能因而導致鹼性正價致突變物與負價蛋白質之離子性相互作用，其原因可能是當在pH2.5時，幾乎所有在酸性胺基酸側鏈上的carboxyl基將不會解離而為正價，此時鹼性正價致突變物與蛋白質間只有最小的靜電交互作用，當pH值由2.5、4.5增至6.8，carboxyl基成為負價時，離子交換於是發生，所以較不易由蛋白質上抽出，而若由pH2.5增至pH10時，則不論最初pH值為何，都能萃取出明顯的致突變性物質。

三、XAD-2 resin分離法：

Bjeldanes, L. F. et al.（1982）(8)以酸水（以HCl滴定至pH2）先使與粗樣品均質化後予以離心，再取上層液以NaOH滴定至中性（pH7），接著通過Amberlite XAD-2 resin管柱，利用非極性的XAD-2 resin來吸附非極性的致突變混合物，以做為最初的過濾。（其萃取步驟見Fig.5.）

Fig 3



Procedures used to investigate the effect of the pH of protein precipitation on the extraction of mutagens from fried fish.

此實驗乃比較四種由煎烤牛肉中萃取出致突變物的方法之總回收率，以分析各種方法之優、缺點，由Table.5.顯示使用XAD-2 resin來分離煎烤牛肉酸萃取液中的致突變物，可以得到比以acetone來萃取的方法（Felton, J. S. et al. 1981）(6)產生將近4倍的反突變菌落數，而甚至以混合溶劑（CH_3OH：CH_2Cl_2：H_2O，45：45：10，V／V／V）來萃取，其回收效率亦仍不及以XAD-2法之一半。

由HPLC層析法之層析圖及安氏檢定法（Ames／Salmonella test）之結果，可知由XAD-2 resin法同樣可分析出他法所萃取出之致突變物（如混合溶劑CH_3OH／CH_2Cl_2／H_2O）（Fig.6.）因此可為一更有效率的分離法，另外，XAD-2法之主要進展在於較以前其他方法（如Table.5.）更能回收酸萃取化合物，所以利用XAD-2法可自食物中依比例增大分離出較大量的致突變物，並且其優點在於花費少、操作簡單且較其他方法快速（只需約一半的時間）。

四、藍棉(blue-cotton)分離法：

藍棉為cotton（Cellulose）與trisulfo-copper-phthalocyanine residues所共價鍵結而成。（如Fig.7.）（製作方法見Ref.(9)）

而致突變性化合物以其多芳香環可在水溶液中與blue cotton充分混合而被cotton上屬於phthalocyanine衍生物之NH－基所吸附，再以溶劑（如ammoniaeal methanol）沖堤出來並予以有效地濃縮，如此重覆（約三次）後，即可得到較純化的多環芳香胺類致突變物。（Hayatsu, H.

Table 4. Comparison of Mutagenic Activities in Organic Extracts of Fried Sole Using Three Different pHs for Protein Precipitation

symbol[a]	pH of partition	no. of revertant colonies[b]	symbol	pH of partition	no. of revertant colonies	symbol	pH of partition	no. of revertant colonies
A	2.5	7 ± 8	A'	4.5	63 ± 6	A''	6.8	62 ± 7
B	10	421 ± 33	B'	10	143 ± 19	B''	10	28 ± 5
A + B		428 ± 34	A' + B'[c]		206 ± 20	A'' + B''[c]		90 ± 9

[a] Symbols refer to different organic extracts in Figure 1. Each extract is equivalent to 10 g of fried fish. [b] *S. typhimurium* strain 1538 with 80 μL of S9/plate. Spontaneous revertants (24) have been subtracted from these numbers. [c] The totals A + B, A' + B', and A'' + B'' are significantly different at the $p = 0.01$ level.

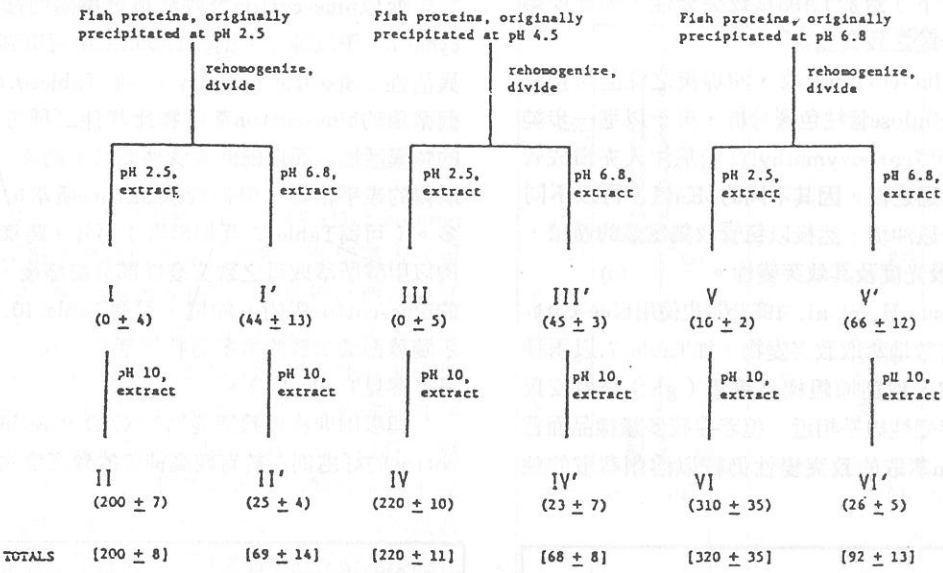


Fig 4. Schematic diagram of the procedure used to reextract fish proteins originally precipitated at pH 2.5, 4.5, and 6.8. The numbers of revertant colonies produced by the various organic fractions (shown in parentheses below each Roman numeral) are for the extract from 10 g of fried sole and have been calculated by using the regression coefficients of linear dose–response curves for each extract. Total revertants obtained by each procedure are shown in brackets at the bottom. *S. typhimurium* strain 1538 with 80 μL of S9 was used for testing of the extracts. The spontaneous revertants (24) are not included.

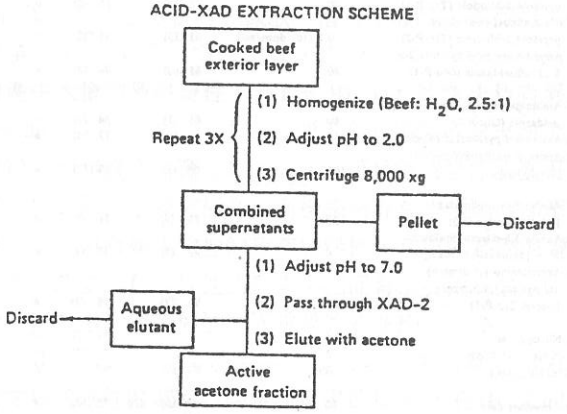


Fig 5. Acid–XAD-2 extraction scheme.

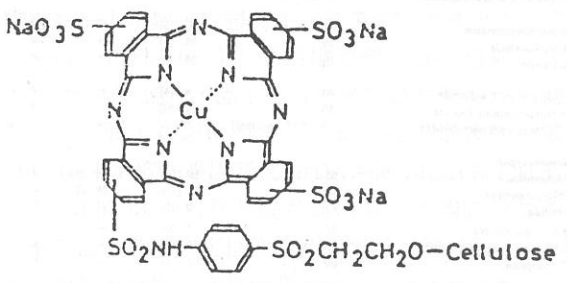
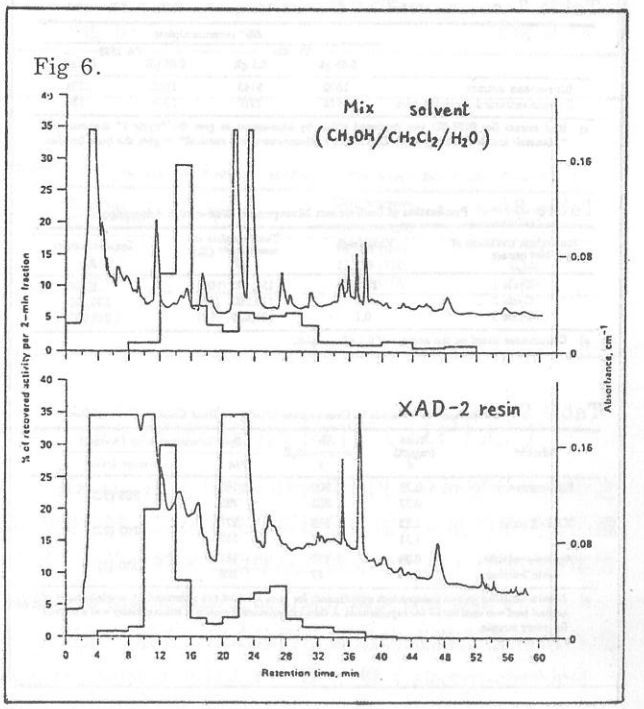


Fig 7. Structure of cotton-bound pigment.

et al. 1983a) (9)

由Table.6.所顯示，具有3個以上芳香環的致突變物幾乎都能有效地被blue-cotton吸附並回收，而少於2個芳香環者，則只有少部分被吸收，但如histidine、oleic acid則幾乎不被blue-cotton所吸收，因此此法可使用來分



離出粗樣品中具frame shift活性的致突變物，因在安氏檢測法（有S9存在下）對於TA98具致突變性，如此即得“經由藍棉濃縮分離之致突變物”。

另外在經過blue-cotton吸收、沖堤後之樣品可接以carboxymethyl cellulose管柱色層分析，再予以進一步純化，乃藉由注射筒將carboxymethyl官能基注入充填成管柱，在加入樣品通過之後，因其不同的pKa值，再以不同組成的溶劑依次予以沖堤，然後以每管收集定量的體積，最後測定各別UV吸光度及其致突變性。

還有，Hayatsu, H. et al. 1983b[10]也使用blue-cotton對牛肉萃取物有效地萃取致突變物，如Table.7.以兩種不同的方式來萃取，取與原粗樣品等量（gE）來做安氏檢定試驗，其致突變性幾乎相近，但若以較多量樣品而言，則以blue-cotton萃取的致突變性仍較以溶劑萃取的鹼層略為高一些。

而以blue-cotton吸收法再重覆兩個循環（cycle 2、cycle 3）予以純化，則在Table.8.中可得到明顯增加的特異活性（Specific activity）。在Table.9.中表示最初一個循環的blue-cotton萃取物比其他二種方法皆具有較高的特異活性，而以總回收活性而言，則與由XAD-2 resin所得的幾乎相等，但仍較以acetone所萃取的鹼層高出甚多。（可與Table.7.互相參考）另外，將樣品換以煎烤牛肉以甲醇所萃取得之致突變性部分濃縮後，經由重覆循環的blue-cotton吸附、沖堤，可得Table.10.之結果，其顯示隨著循環次數的增多而特異活性（rev／mg）亦隨之提高（參見Table.8.）。

與吸附非極性致突變物的XAD-2 resin相比較，blue-cotton的好處則在於有較高純度的致突變物，但是在全部

Table 5.

TA1538 SALMONELLA ASSAY OF EXTRACTS FROM 4 DIFFERENT FRACTIONATION METHODS[a]

Method	Number of revertants per gE[b]	Coefficient of variation
Dilute acid–$(NH_4)_2SO_4$[c]	10–31[d]	–
Acetone[e]	59	±35%
Mixed solvent[f]	98	±46%
XAD-2 resin	235	±34%

[a] For the 3 methods in which bases were separated (dilute acid–$(NH_4)_2SO_4$, acetone, and mixed solvent), only the basic fractions are shown, since the acid and neutral fractions had no mutagenic activity.
[b] Number of revertants per gram fresh weight of uncooked meat (gE) is calculated from the slopes of regression lines in the linear region of the background-corrected dose–response data.
[c] Method of Commoner et al. (1978).
[d] Range of 2 Expts.
[e] Method of Felton et al. (1981).
[f] $CH_3OH:CH_2Cl_2:H_2O$ (45:45:10, v/v/v).

Table 7. Comparison of Methods to Concentrate Mutagens from Difco Beef Extract[a]

	His+ revertants/plate			
	TA 98		TA 1538	
	0.05 gE	0.1 gE	0.05 gE	0.1 gE
Blue-cotton extract	1070	3143	1803	3735
Solvent-extracted basic fraction	1418	2318	2048	3136

[a] Beef extract (lot 0126-02) was processed either by blue-cotton to give the "cycle 1" material (see "Materials and Methods"), or by Commoner's solvent-extraction method[10] to give the basic fraction.

Table 8. Purification of Beef-extract Mutagens by Blue-cotton Adsorption

Blue-cotton treatment of 10 g beef extract	Yield (mg) *A*	Total number of revertants[a] (%) *B*	Specific activity *B/A*
Cycle 1	15.6	150,400 (100)	9,640
Cycle 2	0.3	118,200 (78)	394,000
Cycle 3	0.1	101,800 (68)	1,018,000

[a] Calculations based on the activity of 0.1 gE samples.

Table 9. Comparison of Methods to Concentrate Mutagens from Cooked Ground Beef[a]

Method	Yield (mg/gE) *A*	*His*+ revertants/gE *B*	Specific mutagenicity (rev/mg) *B/A*	Average (ratio)
Blue-cotton	0.27	308	1142	968 (7.5)
	0.33	262	793	
XAD-2 resin	1.23	333	271	292 (2.2)
	1.11	347	312	
Acetone-soluble basic fraction	0.84	127	151	130 (1)
	0.16	17	108	

[a] Results obtained in two independent experiments for each method are presented. A single sample of cooked beef was used for all the experiments. A dose-response relationship of mutagenicity was observed for every sample.

Table 6.

ADSORPTION AND RECOVERY OF COMPOUNDS BY USE OF COTTON BEARING TRISULFO-COPPER-PHTHALOCYANINE ('blue cotton')[a]

Compound	Concentration in saline ($\times 10^{-6}$ M)	% Adsorbed to 'blue cotton' (plain control)	Overall recovery % (plain cotton control)	Assay method
Mutagens				
3-Amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-1)	20	98 (40)	98 (40)	*a*
[^{3}H]3-Amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-2)	0.2 (10^4 dpm/ml)	93 (33)	93 (28)	*r*
2-Amino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole (Glu-P-1)	40	85 (10)	79 (8)	*a*
2-Amino-dipyrido[1,2,-*a*:3′,2′-*d*]-imidazole (Glu-P-2)	80	65 (7)	54 (3)	*a*
2-Amino-9*H*-pyrido[2,3-*b*]indole	100	89 (29)	79 (24)	*a*
2-Amino-3-methyl-9*H*-pyrido-[2,3-*b*]indole	100	88 (45)	88 (37)	*a*
2-Amino-3-methylimidazo-[4,5-*f*]quinoline (IQ)	20	85 (9)	71 (9)	*a*
2-Amino-3,8-dimethylimidazo-[4,5-*f*]quinoxaline (MeIQx)	4	92 (6)	89 (4)	*a*
3-Acetylamino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (Acetyl Trp-P-1)	20	93 (7)	88 (7)	*a*
1-Nitropyrene	2	–	77 (17)	*m*
2-Aminoanthracene	2	–	75 (17)	*m*
Benzo[*a*]pyrene	0.2	97 (61)	60	*f*
9-Aminoacridine	50	96 (15)	75 (12)	*a*
Daunomycin	100	99	65	*a*
[^{14}C]2-Acetylaminofluorene	0.8 (10^4 dpm/ml)	99	96	*r*
2-Aminofluorene	1	–	52 (6)	*m*
Ethidium bromide	50	97 (25)	95[b] (20)	*a*
Quinacrine dihydrochloride	100 (pH 5)	98 (8)	98	*a*
8-Methoxypsoralen	100	60 (5)	59	*a*
Furylfuramide	50	26 (3)	–	*a*
Carbadox	50	36 (3)	–	*a*
Quinoxaline-1,4-dioxide	50	4 (4)	–	*a*
4-Nitroquinoline-1-oxide	50	11 (0)	–	*a*
[^{14}C]Nitrosodimethylamine	0.1 (10^4 dpm/ml)	0	–	*r*
Nonmutagens				
Norharman	50	90 (14)	88 (9)	*a*
Chlorpromazine	40	80 (15)	78 (12)	*a*
Adenine	50	9 (1)	–	*a*
p-Nitrophenol Na	50	5 (0)	–	*a*
4-Nitro-*o*-phenylenediamine	50	19 (4)	–	*a*
Tryptophan	160	3 (1)	–	*a*
[^{3}H]histidine	10 (10^4 dpm/ml)	–	0.6	*r*
	1000 (10^4 dpm/ml)	–	0.7	*r*
Oleic acid Na	10 (pH 9)	7	–	*g*

致突變物的回收率則會降低（見Table.9. A, B／A），不過藍棉仍適用於較廣泛的樣品，懸浮於初樣品之污染物在吸收之前並不須被去除，因此可用以檢測與分離存於天然物、食物、化學產物、尿液、血清及水或空氣等水溶液中的致突變物。

XAD-2 column & Blue cotton extraction之比較：

Inamasu, T., et al.（1988）⑾利用XAD-2 column與Blue cotton萃取未濃縮IQ及其代謝物，此兩種方式可以HPLC分析比較放射性元素^{14}C標定追蹤的總回收率及其主要代謝物之結果。Fig.8.、Fig.9.在HPLC分析方面，有3個主要波峯在極性區域而另2個鄰近的波峯則在非極性區域，給予老鼠^{14}C－IQ後，則在其尿中可發現放射性存在，XAD-2 resin約吸收65％（45＋20％）放射性（見Table.11.），而blue cotton則約吸收23％的放射性（見Table.12.）。兩種方法乃皆由混合代謝物中有效的吸收並釋放出相關非極性化合物，但同樣都不能保留更多極性代謝物，因此XAD-2管柱和blue cotton的吸收技術都可以使用於由較大量的水溶液中濃縮出非極性致突變物。

食物樣品附加致突變性的偵測：

Becher, G. et al.（1988）⑿在探討肉類中加入creatine後所發生之影響時，採取不捨棄殘渣的方式而仍再將殘渣以acetone予以均質化後，再以CH_2Cl_2來萃取，而仍會產生大量的致突變性（見Fig.10.），其作法乃有異於Bjeldanes, L. F. et al.（1982）⑻於經酸水抽出致突變物後，即將殘渣捨棄（Fig.5.），可見當時之作法極可能還漏失一部分之致突變物，而後來再經加以丙酮附加萃取後，之所以會產生附加的致突變活性，乃可能是因丙酮對於肉塊的滲濕具有較佳的效果，因而有助於更多酸水的回收，以致增加致突變性，而重要的是在Knize, M. G. et al.（1988）⒀的煎肉研究中，發現以丙酮所萃取出來的附加致突變性區域與以酸水所萃得的區域，在HPLC的分析圖上是在同樣的位置，因此表示此附加致突變物並非其他不同的致突變物。還有在此研究中還將殘留於鍋底之煎肉剩餘物取下萃取，而將煎牛、雞肉致突變性分析分成三方向進行（見Fig.11.），同樣地都經XAD-2管柱分離後，進入HPLC的純化，圖中值得一提的是右側一鍋底殘餘物的萃取方面，在以methylene chloride萃取後的鹼層再以butanol來萃取，是因其比methylene chloride更具有效率。於Table.13.中可見不論是雞肉或是牛肉在附加的丙酮萃取方面，都有一定程度的致突變量（約15％），且在鍋底殘餘物的萃取上更是約有30%的致突變性產生，可見此兩方向致突變物的抽取乃值得注意，並不可隨意捨去，以免損失一部分致突變物。

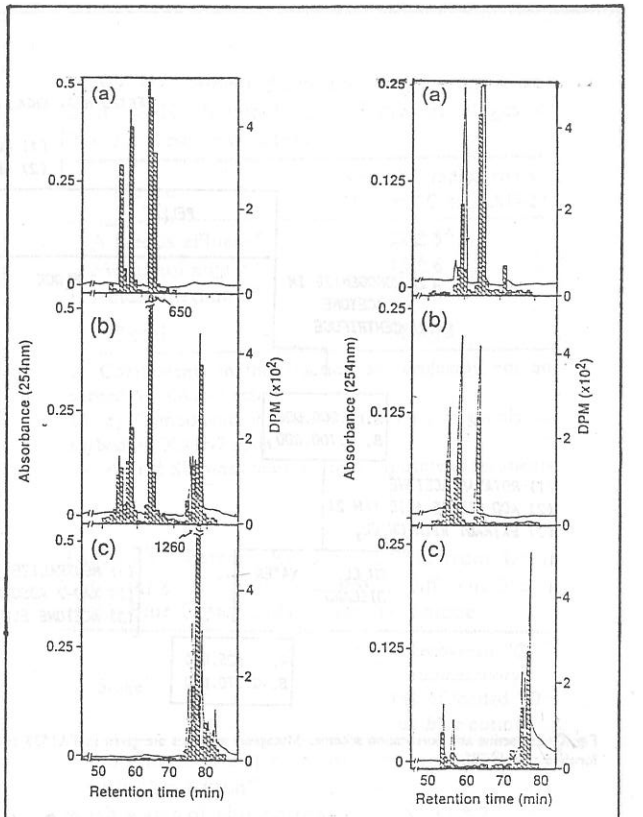


Fig. 8 HPLC chromatograms of the XAD-2 column eluates following treatment of urine for extraction of IQ metabolites. a) Aqueous effluent. Note the presence of the polar conjugated metabolites. b) Methanol eluate. Note that this fraction contained polar conjugates and also nonpolar compounds. c) Acidic methanol eluate. Note that this eluate contained exclusively the nonpolar fraction

Fig. 9. HPLC chromatograms of the urine fractions treated with blue cotton. a) Urine sample after treatment with blue cotton. Note that the blue cotton absorbed very little of the polar fraction and failed to absorb some of the nonpolar metabolites. b) Aqueous phase used for washing the blue cotton soaked in urine. c) Eluate obtained from ammoniacal methanol treatment of the blue cotton soaked in urine. Note that this method leads to the absorption of very little of the polar metabolites but appears to absorb the nonpolar metabolites, including IQ itself.

Fig 8. Fig 9.

Table 10. Isolation and Purification of Mutagenic Components from Cooked Ground Beef

Blue-cotton extraction cycle	Yield (mg)	Total revertants and raw beef equivalent (gE)	Specific activity (rev/mg)
Cycle 1	13.2	94,320 (180)	720
Cycle 2	0.8	51,720 (174)	6,500
Cycle 3	0.3	50,570 (168)	17,000

五、高效液相層析法(HPLC)：

Kasai, H.（1979）⒁利用HPLC分析烹煮魚肉中之致突變物時，以在原來分析樣品中加入可能的致突變物標準品，然後比較未加標準品之樣品分析圖，若增強波峯恰為致突變物之波峯時，則可確定為此致突變物。

至於一般在HPLC分析所採用的偵測方法有紫外光（ultraviolet）（UV）、電化學（electro-chemical）

Fig 10.

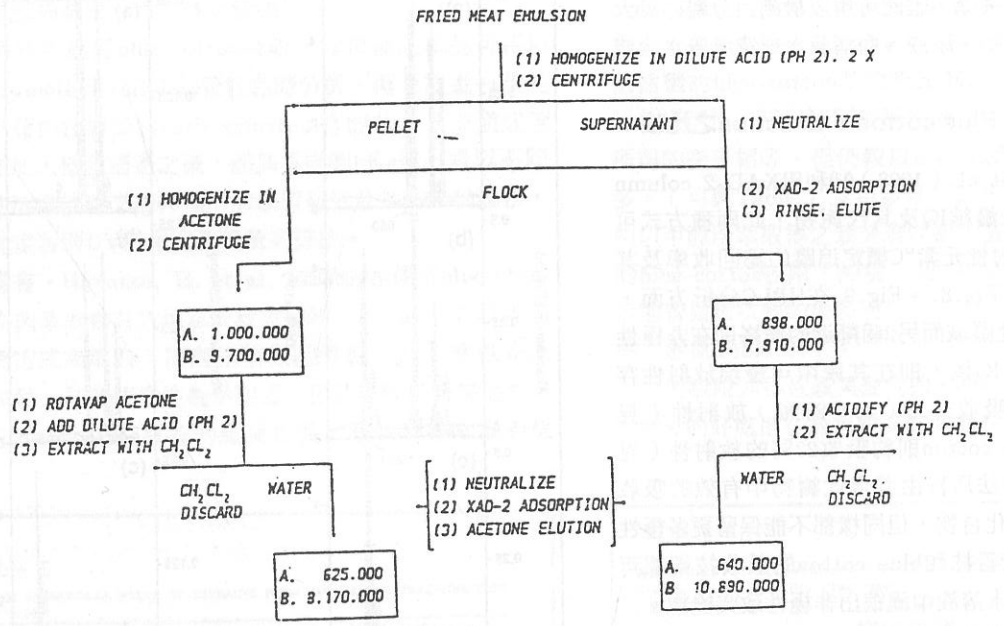


Fig.10. Extraction and purification scheme. Mutagenic activities are given in TA1538 revertants/kg fresh wt of the regular sample (A) and for the creatine fortified sample (B).

Fig 11.

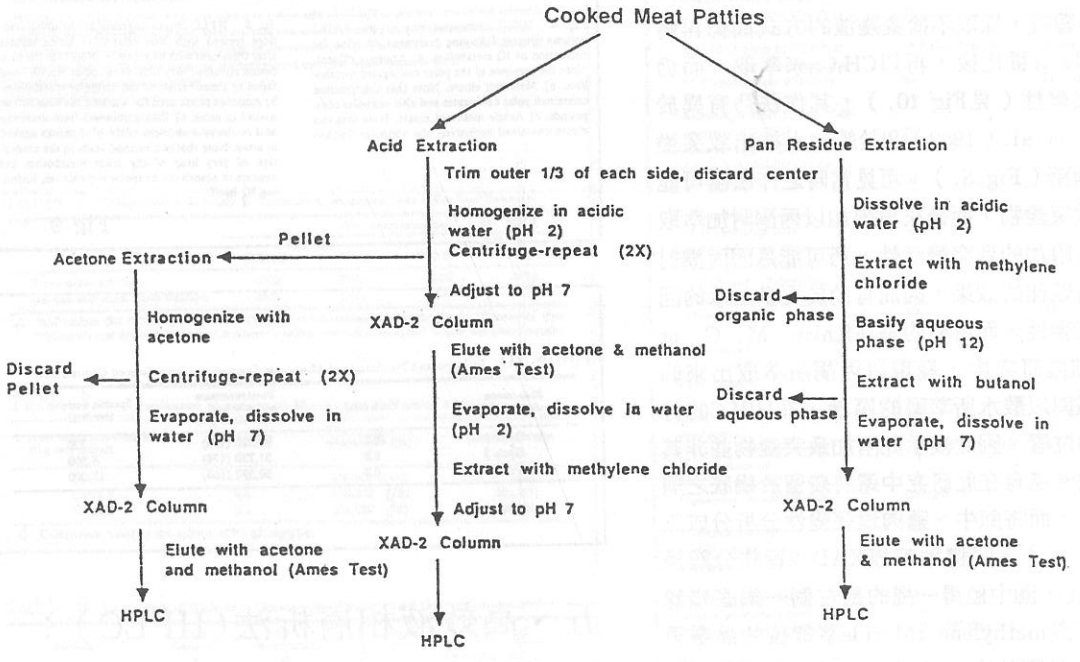


Fig.11 Extraction scheme for patties and pan residues from both fried chicken and beef.

(EC)及螢光(fluorescence)偵測等，至於各種檢測方式間的差異如何，即需加以比較。Grivas, S.及Nyhammar, T.(1985)[15]就將紫外光及電化學偵測法等予以比較。在Table.14.中可見電化學的液相層析偵測(LCEC)很明顯地比紫外光偵測更為敏感(LCEC：0.5～1.5 pmoles, LCUV：2.5 pmoles)但紫外光的偵測極限(2.5 pmoles)

很少能達到因為由於有不純物質而引起波峯的重疊（overlapping），所以若使用電化學偵測器，則不純物質在操作電位並不會被氧化，因此不會掩蓋IQ化合物之偵測。當敏感度具有判斷力時，電化學偵測的選擇性即相對地有價值，而其選擇性要視某些分子在較低電位的氧化或還原的情況而定，理論上，給予一夠高的電位則很多分子都能氧化，而實際上，移動相可以限制電極電位的範圍，因此在可以偵測的範圍內許多分子並不是都能在電化學活化，所以可以靠改變電位來調節選擇性，可知，電化學偵測的優點乃是在較低的偵測極限下有較高的敏感度，不過其缺點則在於敏感度的增高會造成干擾而不具穩定性。

另外，液相層析（LC）更可和質譜（MS）結合而進一步分析，Yamaizumi, Z. et al.（1986）[16]以放射性〔Me－$^2H_3$〕標定的異構物加入後，能在HPLC準確偵測色層分析滯留度（K′）或直接結合液相層析一質譜（LC／MS），分析質子化分子的離子與內加標準品的反應比例之測量，而分離並定量致突變物。其步驟見Fig.12.，其加入放射性標定致突變物為內標準品之步驟是在於甲醇萃取之後、blue cotton萃取之前，而LC／MS分析見於Fig.13、14。還有Juresky, R. J. et al.（1988）[17]也以此方式由不同樣品中分析出不同致突變物，其步驟見Fig.15.，其中加入放射性標定致突變物為內標準品之步驟是在甲醇萃取之前，此可與Fig.12.相比較，而其LC／MS之分析見於Fig.16.，如此即可確定其致突變物。

而最近Gross, G. A.（1990）[18]，提出在食物產物中定量致突變性異環胺類的簡易法，乃以兩種微量分析之固相萃取法來確定加熱肉類產物的致突變性異環芳香胺類。其一為Copper phthalocyanine（CPC）雙管萃取，乃結合diatomaceous earth管柱及CPC衍生Sephasorb HP，

Table 11. Percent Recovery ^{14}C-IQ from Urine with ^{14}C-IQ Metabolites at Different Stages of XAD-2 Resin Extraction

Elution	Recovered ^{14}C radioactivity (% of loaded ^{14}C on XAD-2)
Aqueous effluent[a]	32±5[d]
Methanol eluate[b]	45±6
Acidic methanol[c]	20±3
Total	98±13

a) Components in this fraction are originally not adsorbed on XAD-2 resin.
b), *c*) Components in these fractions are originally adsorbed on XAD-2 resin.
d) Mean±SD was obtained from triplicate experiments.

Table 12. Percent Recovery of ^{14}C from Urine Containing ^{14}C-IQ Metabolites at Different Stages of the Blue Cotton Adsorption Technique

Stage	Recovered ^{14}C radioactivity (% of loaded ^{14}C on blue cotton)
Urine sample after treatment with blue cotton[a]	62±5[d]
Wash water of blue cotton soaked in urine[b]	13±2
Ammoniacal methanol extract from blue cotton[c]	23±3
Total	99±7

a), *b*) Components in these fractions are originally not adsorbed on blue cotton.
c) Components in this fraction are originally adsorbed on blue cotton.
d) Mean±SD was obtained from triplicate experiments.

Table 13.

Distribution of mutagenic activity and effect of creatine for cooked chicken and beef

	Endogenous creatine[a]	Added creatine[b]	Ratio of added creatine:endogenous creatine
Chicken			
Acid extraction	44% (69)[c]	58% (78)	2.6 (2.8)
Acetone extraction	17% (7)	13% (3)	1.5 (1.1)
Pan residue	39% (24)	29% (19)	1.4 (2.0)
Total TA1538 revertants	120 000 (250 000)	230 000 (600 000)	2.0 (2.4)
Beef			
Acid extraction	61% (72)	57% (71)	2.7 (3.5)
Acetone extraction	16% (7)	16% (7)	2.9 (3.6)
Pan residue	23% (21)	27% (22)	3.4 (3.5)
Total TA1538 revertants	150 000 (260 000)	440 000 (890 000)	2.9 (3.5)

[a]Natural creatine level = 0.4–0.5%; chicken 0.50% (3.8 μmol/g); beef 0.41% (3.1 μmol/g).
[b]4% creatine by fresh weight added to patty before frying.
[c]Replicate experiment in parentheses.

然後可在Sephasorb HP沖堤得致突變物，許多屬於ppb級量的致突變物，如MeIQx、IQ、MeIQ、PhIP、$A_{\alpha}C$、Trp-P-1、Trp-P-2、harman及norharman等，皆可以同時使用HPLC以紫外光來偵測，而另一方法為採取propyl sulfonyl silica gel（PRS）雙管萃取為一個步驟的沖堤方法，為結合diatomaceous earth管柱及PRS之方式，而適合於確定MeIQx、IQ、Glu-p-1、Glu-p-2等致突變物，例如可將7.8－D：MeIQx當作內標準品（internal standard）來偵測判別，且一般只需約4克或較少的樣品來分析即可。而經估算其胺類的回收率約介於46%和83%

Fig 13.

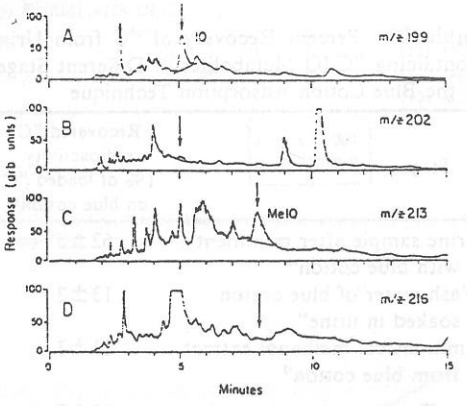


Fig. 13. LC/MS analysis of purified extract of salmon flesh without isotopically labeled internal standards. Conditions in experimental section. Profiles are for MH^+ ions monitored simultaneously in a single analysis. Arrows show expected elution positions for reference compounds. (A) IQ, (B) $[^2H_3]$IQ, (C) MeIQ, (D) $[^2H_3]$MeIQ.

Fig 14.

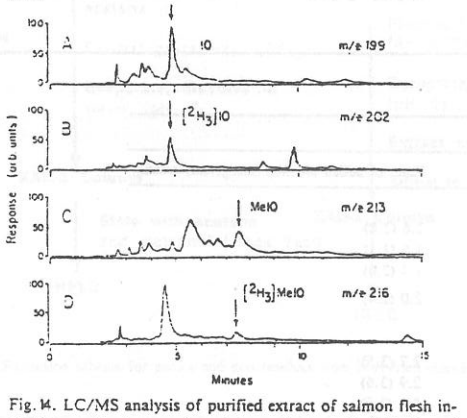


Fig. 14. LC/MS analysis of purified extract of salmon flesh including stable isotope internal standards, [*Me*-$^2H_3$]IQ and [*Me*-$^2H_3$]MeIQ. Conditions in experimental section. Details same as in Fig.13.

Fig 12.

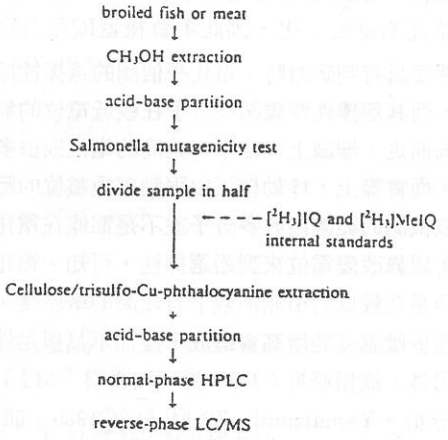


Figure 12 Scheme for extraction of mutagens for analysis by LC/MS.

Table 14.

COMPARISON OF DETECTION LIMITS (pmoles)

Compound	LCUV	LCEC
IQ	2.5	1.1
MeIQ	2.5	1.5
MeIQx	2.5	0.7
4,8-DiMeIQx	2.5	0.8

Table 15.

Extraction parameters

	Preparation		
	I	II	III
Extrelut column			
Sample quantity (g)	0.5–1	4.0	3.0
Amount 1 N NaOH (ml)	2	10	5.5
Extrelut type	3	20	[a]
Extraction volume (ml DCM)	15	40	40
CPC method			
CPC Sephasorb gel volume (ml)	1.3	2.6	2.6
Desorption volume (ml 15% MeOH/NH_3/DCM)	7	15	15
Sephasorb gel volume (ml)	1	2	2
Wash step (ml 25% MeOH/H_2O)	5	10	10
Desorption volume (ml MeOH/NH_3)	10	15	15
PRS method			
Sorbent quantity (mg)	100	500	500
NP–RP conversion (ml 0.01 N HCl)	3	6	6
Wash step (ml MeOH/0.1 N HCl 60:40)	4	20	20
Desorption volume (ml MeOH/NH_3 9:1)	3	10	–
Transfer solution (ml 0.5 N NH_4Ac)	–	–	20

[a] Extrelut-20 refill units.

Fig 15.

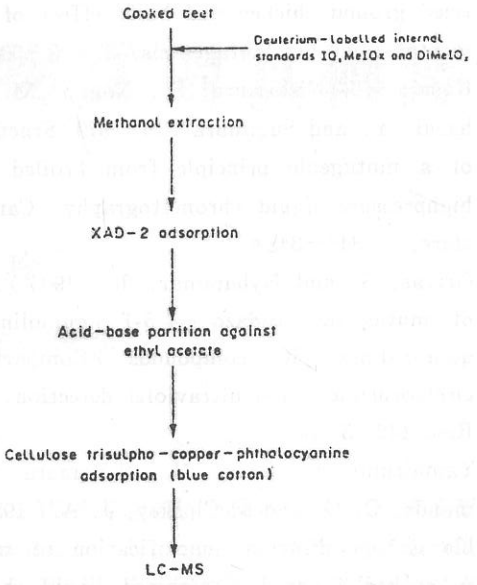


Fig. 15.Purification scheme for heterocyclic amines found in cooked beef products.

Fig 16.

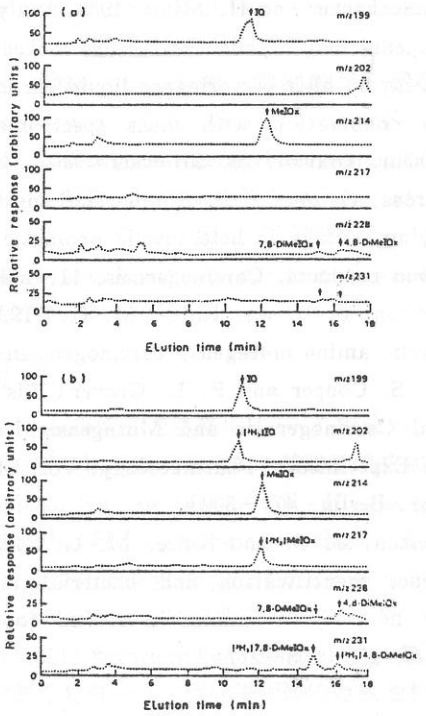


Fig.16. LC-MS analysis of purified bacterial-grade beef extract (a) without or (b) with isotopically-labelled internal standards. Profiles are for MH^+ ions with arrows marking the expected elution positions for IQ, $[^2H_3]$IQ, MeIQx, $[^2H_3]$MeIQx, DiMeIQx and $[^2H_3]$DiMeIQx isomers. $[^2H_3]$IQ and $[^2H_3]$MeIQx were added to beef extract at levels of 50 ng/g, and $[^2H_3]$DiMeIQx isomers were added at 10 ng/g. Analysis was performed with a C-18 reverse-phase column as described in the Materials and Methods section.

之間，另外在偵測極限上，為低於ppb（1／10^9）的範圍，其偏差係數則在5%到18%之間，此兩方法之優點乃在於省時，且專為只需較少量樣品轉換、蒸發的步驟而設計，因此可使致突變物的操作損失量減至最小，而使有限的致突變物更容易偵測到，不過其費用的偏高，乃為其缺點。

而CPC法和PRS法都具有三種配制的變數（preparative variants）：Ⅰ、Ⅱ及Ⅲ（見Table.15.），可配合於不同種類及數量的樣品，變數Ⅰ和Ⅱ是屬於微量及小量樣品的參數，使可偵測之肉萃取物分別大至1克和4克左右；而變數Ⅲ，則是為了較大量的煎肉萃取之用。另外，可分別地以各種萃取媒介變數（parameters）如樣品數量、溶解溶劑、pH值、以及萃取所需溶劑的數量等來試驗，可以得到最合適的條件（參見Table.15.），這些也可以加入放射性標定之標準品（如〔2－^{14}C〕MeIQx）來予以確定。

六、結語：

累積了十餘年的致突變物萃取、分離、純化等分析技術，由於前人在食物致突變成份的研究努力，使得現今致突變物的找尋、偵測、確定等方法，似乎有擺脫以往傳統技術的趨勢，其目標不外乎在追求研究更快、更簡易且更為精確的分析方向，雖然所採取的材料、設備日異更新，結果也愈來愈精美，然而所根由的基本理論仍舊大為可尋，因此若在日後要再求新求變的同時，也不要遺忘最初的原始技術，如此才能相輔而相成。

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