This is to certify that the

dissertation entitled

Inhibition of heterocyclic aromatic amine formation in fried ground beef patties by organosulfur compounds in garlic

presented by

Hanseung Shin

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Food Science

Major professor

Date Dec. 6, 2001

INHIBITION OF HETEROCYCLIC AROMATIC AMINE FORMATION IN FRIED GROUND BEEF PATTIES BY ORGANOSULFUR COMPOUNDS IN GARLIC

By

Hanseung Shin

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Food Science and Human Nutrition

2001

ABSTRACT

INHIBITION OF HETEROCYCLIC AROMATIC AMINE FORMATION IN FRIED GROUND BEEF PATTIES BY ORGANOSULFUR COMPOUNDS IN GARLIC

By

HANSEUNG SHIN

The effect of organosulfur compounds on heterocyclic aromatic amine (HAA) formation and overall mutagenicity in fried beef patties was studied. Organosulfur compounds (0.67 mmol) were added directly to 100g of ground beef and fried at 225°C for 10 min per side. HAAs were isolated by solid phase extraction and quantitated by high performance liquid chromatography (HPLC). Concentrations of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*] pyridine (PhIP), the major HAA in muscle foods, were reduced by 81 and 69% by diallyl disulfide (DAD) and dipropyl disulfide (DPD), respectively, while cysteine and cystine were less effective inhibitors.

In a second series of experiments, the effect of organosulfur compounds on HAA formation in fried ground beef patties and mutagenicity was evaluated by HPLC analysis and the Ames *Salmonella typhimurium* assay. The greatest inhibition of HAA formation was observed by DAD and DPD, with reductions of 78 and 70%, respectively. The overall mutagenicity of the fried beef patties was reduced 75 and 65% by DAD and DPD, respectively. The measured mutagenicity in fried beef patties was quite similar to the mutagenicity values calculated from the determined concentrations of HAAs.

A series of model system studies were conducted to more completely understand the mechanism by which HAA formation is inhibited by sulfur compounds. The concentrations of PhIP increased 3-4 fold when glucose was added to the model system containing phenylalanine and creatinine. These studies confirmed glucose as an important contributor to HAA formation. Organosulfur compounds and sodium bisulfite effectively inhibited HAA formation in model systems containing phenylalanine, glucose, and creatinine. However, these compounds had no effect on HAA formation in the model systems that did not contain glucose. A possible mechanism of HAA inhibition by DAD and DPD in model systems containing glucose could be through their interaction with glucose. A number of sulfur-containing compounds such as tetrahydrothiophene-3-one (THT) and tetrahydrothiophene (THP) were produced by heating glucose and DAD at 180°C for 30min. However, THT and THP had no effect on HAA formation in the various model systems, regardless of whether glucose was present or not. THT and THP are merely reaction products between glucose and DAD and do not influence HAA formation.

While these experiments point to a competitive reaction between organosulfur compounds and amino acids for glucose, the mechanism by which these compounds inhibit HAA formation is still not fully clarified. However, the observation that DAD has no effect on HAA formation in model systems without glucose provides supporting evidence that the interaction of DAD with glucose is a possible key element in its inhibition of HAA formation. It is also apparent that the products of interaction of glucose and DAD are not directly involved in the inhibition process.

Dedicate to my parents for helping

me find my way in life

ACKNOWLEDGEMENTS

I wish to acknowledge my sincere gratitude to my two advisors, Dr. Gale M. Strasburg and Dr. J. Ian Gray, for their patience, support and encouragement throughout my studies at Michigan State University. Dr. Gray's enthusiasm for scientific research and motivation for independent thinking will always be an inspiration to me. I also appreciate very much Dr. Gray's patience in helping me develop my dissertation. Dr. Strasburg has been an excellent advisor and I cannot thank him enough for all the help and guidance he has provided me. Appreciation is also extended to Drs. A.M. Booren and Muraleedharan G. Nair for serving on my guidance committee and all the helpful meetings and suggestions for my research.

Sincere thanks and appreciation are given to Dr. Enayat Gomaa for her assistance with the experimental design of the studies and with the extraction and HPLC analyses. I wish to thank my laboratory colleagues, John Rodgers, Aziz Awad, Wen Chiang, Hyojung Yoon, Andrea Molengraft, and Vareemon Tuntivanich for their invaluable help and encouragement. I also appreciate the helpful suggestions of a number of faculty and staff members in the Department of Food Science and Human Nutrition.

My great appreciation goes to my dear parents Drs. Hyosun Shin and Yangja Yoo who have offered me tremendous support, encouragement, and love throughout my years at Michigan State University. I also thank my two sisters for their concern and support during the completion of this dissertation. Finally, I would like to offer my special thanks and love to my wife, Sojung Seo, for her support and help along the way.

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INTRODUCTION

Heterocyclic aromatic amines (HAAs) are commonly found in meat and fish products cooked at temperatures greater than 150 °C. These compounds have been classified into two categories, pyrolytic mutagens and thermic mutagens, based on their temperatures of formation. Pyrolytic mutagens are formed when proteins and/or amino acids are heated to high temperatures (>300 °C) and are characterized by a pyridine ring with an amino group attached (Skog, 1993; Wakabayashi and Sugimura, 1998). Thermic mutagens are formed at lower temperatures (<300 °C), and several have been identified in cooked muscle foods. These compounds, also called aminoimidazoazaarenes, can be broken down into four major categories: quinolines, quinoxalines, pyridines, and furopyridines. The most commonly found HAAs in foods are IQ (2-amino-3-methylimidazo[4,5-*f*]-quinoline); MeIQ (2-amino-3,4dimethylimidazo[4,5-*f*]-quinoline); MeIQx (2-amino-3,8 dimethylimidazo [4,5-*f*]-quinoxaline); 4,8 DiMeIQx (2-amino-3,4,8 trimethylimidazo[4,5-*f*]-quinoxaline); and PhIP (2-amino-1-methyl-6-phenylimidazo [4,5-*b*]-pyridine) (Knize et al., 1999; Skog, 1993; Wakabayashi and Sugimura, 1998).

Many of the HAAs isolated from foods have been shown to be mutagenic by the Ames *Salmonella typhimurium* mutagenicity assay (Felton et al., 1997) and by mammalian cell culture studies such as those employing Chinese hamster ovarian cells (Holme et al., 1989). Mutagenicity varies widely among individual HAAs, and has been reported to be as high as 661,000 revertants/μg toward *S. typhimurium* TA98. Aflatoxin B1, a well-documented carcinogen, causes only 6,000 reverents/μg under the same assay conditions. It has been reported that HAAs, when added to diets, will

produce carcinogenic lesions in mice and rats (Esumi et al., 1989). Because HAAs are found in a variety of cooked foods which constitute a major part of the diet of the U.S. population, they are considered to be potential risk factors for human health (Hirose et al., 1999).

The precursors of HAAs in cooked meat products are thought to be creatine/creatinine, amino acids and sugars (Jägerstad et al., 1983a). It has been suggested that HAA formation follows the Maillard reaction through the generation of vinylpyrazine, vinylpyridine and aldyhydes (Jägerstad et al., 1983b). Factors influencing HAA formation include the temperature, time, and method of cooking, as well as the concentrations of precursors present in the food (Knize et al., 1994; Skog et al., 1992).

Several approaches to decreasing HAA formation in food systems have been suggested. Concentrations of HAA precursors in meat patties (creatine, amino acids and sugar) are reduced by microwave pretreatment of the patties before frying (Felton et al., 1992). Addition of glucose or lactose at levels ranging from 2 to 4 percent will reduce the overall mutagenicity of cooked ground meat (Skog et al., 1992). Food ingredients, such as vitamin E and tea phenolic antioxidant compounds, will reduce HAA formation in meat (Balogh et al., 2000; Weisburger et al., 1994). Soy protein concentrates (Wang et al., 1982) and the marinating of meats before cooking (Salmon et al., 1997) will also inhibit HAA formation.

Sulfur compounds have been reported to provide various health-promoting benefits including antiplatelet activity (Bordia et al., 1998), antiproliferative activity against human colon tumor cells (Knowles and Milner, 1998), and the lowering of

plasma and liver cholesterol levels (Omkumar et al., 1993). Several studies have revealed the ability of selected classes of sulfur compounds to inhibit HAA formation in meats and model food systems. Addition of sulfur compounds to an aqueous pork extract reduced the formation of Maillard reaction products with a concomitant decrease in mutagenicity (Tsai et al., 1996). Trompeta and O'Brien (1998) demonstrated that sulfur compounds such as glutathione, L-cysteine, L-cystine, and deoxyalliin inhibited the formation of HAAs in model systems containing glucose, glycine, and creatinine. Furthermore, the addition of onion juice to ground beef reduced HAA formation (Kato et al., 1998).

The mechanism(s) by which sulfur compounds inhibit HAA formation during the cooking of meats has/have not been fully investigated. Several investigators have proposed possible mechanisms including the inhibition of the Maillard reaction and the suppression of free radical formation by thiol trapping (Friedman and Molnar-Perl, 1990; Tsai et al., 1996).

This study is based on the hypothesis that sulfur compounds, when added to model systems containing creatinine, amino acids and glucose, and ground beef patties, will inhibit HAA formation through selected competitive reactions. This hypothesis is based on the premise that the Maillard reaction plays an integral role in the formation of HAAs. To test this hypothesis, a number of objectives were developed to not only confirm the inhibitory role of selected sulfur compounds in meat and model systems, but also to gather data that will lead to a fuller understanding of HAA inhibition by sulfur compounds.

Specific objectives of the study are as follows:

1. To evaluate the effect of selected organosulfur compounds present in garlic on HAA formation in fried ground beef patties.

2. To investigate the relationship between the reduction of specific HAA compounds and overall mutagenicity of the cooked meat system as determined by the Ames *Salmonella typhimurium* assay.

3. To conduct selected experiments that will lead to a more complete understanding of the inhibition of HAA formation by sulfur compounds.

CHAPTER ONE

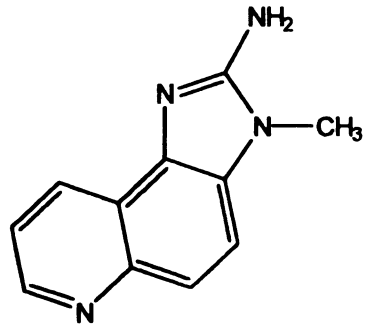
LITERATURE REVIEW

Heterocyclic Aromatic Amines in Meat Systems

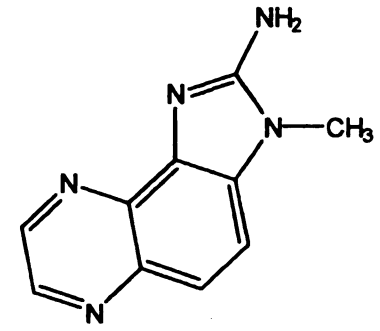
Heterocyclic aromatic amines (HAAs) have been classified into two categories based on their temperatures of formation: *pyrolytic mutagens and thermic mutagens*. Pyrolytic mutagens are formed when proteins or amino acids are heated to temperatures in excess of 300°C and are characterized by a pyridine ring with an amino group attached (Skog, 1993). Thermic mutagens are formed at lower temperatures (<300 °C), and several have been identified in cooked meat and fish. These mutagens, also called aminoimidazoazaarenes, can be broken down into four categories: *quinolines, quinoxalines, pyridines, and furopyridines*. Several have been isolated and identified in cooked meat, fish, and food-grade beef extracts (Table 1). The most commonly formed HAAs in meat products are IQ (2-amino-3-methylimidazo[4,5-*f*]-quinoline); MeIQ (2-amino-3,4dimethylimidazo[4,5-*f*]-quinoline); MeIQx (2-amino-3,8 dimethylimidazo [4,5-*f*]-quinoxaline); 4,8 DiMeIQx (2-amino-3,4,8 trimethylimidazo[4,5-*f*]-quinoxaline); and PhIP (2-amino-1-methyl-6-phenylimidazo [4,5-*b*]-pyridine) (Skog, 1993). Their structures are shown in Figure 1.

Quinolines

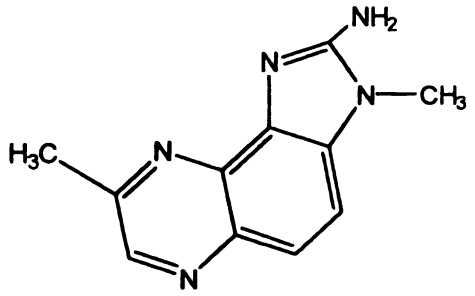
Two imidazoquinoline compounds, IQ and MeIQ, were first identified by Kasai et al. (1980) in broiled sun-dried sardines. These compounds have been subsequently



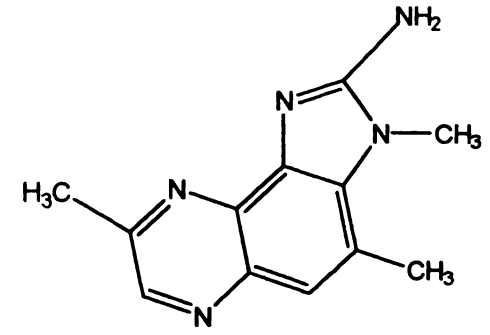
IQ



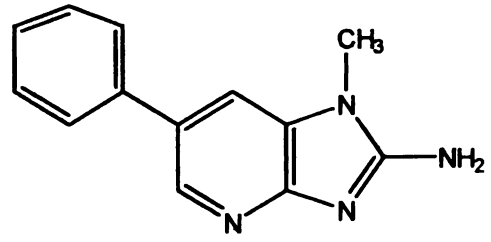
IQx



MeIQx



4,8-DiMeIQx



PhIP

Figure 1. Chemical structures of some HAAs found in cooked foods (Skog, 1993).

Table 1. Heterocyclic aromatic amine content of cooked foods.

Food type	Mutagen	Amount[1]	Temp[2]	Time[3]	Weight[4]	Ref[6]
BEEF						
Fried	MeIQx	0.5			0	43
	4,8-DiMeIQx	3.9	275	15	0	38
	8-MeIQx	12.3	200	15	0	38
	8-MeIQx	4.0	150	6	0	25
	IQ	1.9	200	15	0	38
Heated Bouillon						
	4,8-DiMeIQx	0.3			0	33
	8-MeIQx	0.6			0	33
	PhIP	0.3			0	33
Boiled Extract						
	4,8-DiMeIQx	28.0			1	14
	4,8-DiMeIQx	0.0-3.7			1	39
	4,8-DiMeIQx	0.0-4.4			1	9
	4,8-DiMeIQx	0.0			1	33
	4,8-DiMeIQx	2.5-4.9			1	10
	7,8-DiMeIQx	0.0			1	39
	8-MeIQx	28.0			1	14
	8-MeIQx	3.1			1	41
	8-MeIQx	20.5			1	38
	8-MeIQx	8.5-30.0			1	9
	8-MeIQx	23.0-69.0			1	40
	8-MeIQx	0.0-44.0			1	10
	8-MeIQx	0.6			1	33
	8-MeIQx	3.1			1	43
	8-MeIQx	11.7-36.4			1	39
	AαC	0.0			1	10
	IQ	0.0			1	41
	IQ	0.0			1	14
	IQ	0.5			1	36
	IQ	4.8-6.2			1	40
	IQ	0.0-6.2			1	39
	IQ	0.0-8.0			1	9
	PhIP	3.62			1	15
	PhIP	0.0			1	9
	PhIP	0.0			1	19

Food type	Mutagen	Amount[1]	Temp[2]	Time[3]	Weight[4]	Ref[5]
	PhIP	0.0			1	33
	4'-OH-PhIP	21.0	15	60	1	27
Heated Extract						
	4-CH_2OH-8-Mex	6.2	121	60	1	24
	4-CH_2OH-8-Mex	6.7	160	300	1	24
	4-CH_2OH-8-Mex	7.2	200	300	1	24
Flavor						
	4,8-DiMeIQx	0.0			1	16
	4,8-DiMeIQx	0.0			1	16
	MeIQx	0.0-12.5			1	16
	MeIQx	0.0-4.4			1	16
Roasted	4,8-DiMeIQx	0.0			1	16
	MeIQx	0.0-4.4			1	16
Grilled	4,8-DiMeIQx	0.0			1	16
	MeIQx	0.0			1	16
GROUND BEEF						
Charbroiled	4,8-DiMeIQx	0.2		6	1	17
	4,8-DiMeIQx	0.1		10	1	17
	MeIQ	0.0		6	1	17
	MeIQ	0.4		10	1	17
	MeIQx	1.0		6	1	17
	MeIQx	0.4		10	1	17
	MeIQx	0.2-1.8			1	47
	IQ	0.0		6	1	17
	IQ	0.1		10	1	17
	PhIP	0.0		6	1	17
	PhIP	5.2-18.4			1	47
Broiled	IQ	0.5			1	44
Grilled	AαC	0.0	270	3	1	12
	AαC	0.0	270	5	1	12
	AαC	0.0	270	7	1	12
	4,8-DiMeIQx	0.0	270	3	1	12
	4,8-DiMeIQx	0.0	270	5	1	12
	4,8-DiMeIQx	0.0	270	7	1	12
	MeIQx	0.8	270	3	1	12
	MeIQx	2.0	270	5	1	12
	MeIQx	1.3-4.6			1	47
	PhIP	0.7	270	3	1	12
	PhIP	1.4-4.8	270	5	1	12
	PhIP	16.8			1	47

Food type	Mutagen	Amount[1]	Temp[2]	Time[3]	Weight[4]	Ref[5]
	PhIP	0.0	270	7	1	12
Fried	AαC	21.0	277	6	1	37
	DiMeIQx	4.5	277	6	1	37
	DiMeIQx	4.8	225	10	1	49
	4,8-DiMeIQx	0.5	300	6	0	7
	4,8-DiMeIQx	3.9	275	15	0	39
	4,8-DiMeIQx	0.0	275	5	0	39
	4,8-DiMeIQx	0.0	275	10	0	39
	4,8-DiMeIQx	0.5-1.2	200		2	32
	4,8-DiMeIQx	0.12			0	35
	4,8-DiMeIQx	0.3			0	25
	4,8-DiMeIQx	0.0-0.28			2	5
	4,8-DiMeIQx	0.54	250	12	1	25
	4,8-DiMeIQx	0.1	150	6	0	26
	4,8-DiMeIQx	0.0	150	2	0	26
	4,8-DiMeIQx	0.0	150	4	0	26
	4,8-DiMeIQx	0.7	150	10	0	26
	4,8-DiMeIQx	0.0	190	2	0	26
	4,8-DiMeIQx	0.10	190	4	0	26
	4,8-DiMeIQx	0.55	190	6	0	26
	4,8-DiMeIQx	2.6	190	10	0	26
	4,8-DiMeIQx	0.15	230	4	0	26
	4,8-DiMeIQx	0.25	230	6	0	26
	4,8-DiMeIQx	9.35	230	10	0	26
	4,8-DiMeIQx	0.7	225	6	1	29
	4.8-DiMeIQx	3.1			1	25
	7,8-DiMeIQx	0.0	275	5	0	39
	7,8-DiMeIQx	0.0	275	10	0	39
	7,8-DiMeIQx	0.7	275	15	0	39
	4-MeIQ	0.1	300	6	0	7
	MeIQx	1.0	250	6	1	6
	MeIQx	16.4	277	6	1	37
	MeIQx	1.0	300	5.5	0	4
	MeIQx	0.0			0	19
	MeIQx	0.0-0.68			2	5
	MeIQx	0.3			0	43
	MeIQx	1.3-2.4	200		2	32
	MeIQx	5.8	225	10	1	49

Food type	Mutagen	Amount[1]	Temp[2]	Time[3]	Weight[4]	Ref[5]
	MeIQx	2.7	275	5	0	39
	MeIQx	4.2	275	10	0	39
	MeIQx	12.3	275	15	0	39
	MeIQx	0.5-1.5			0	40
	MeIQx	1.3-1.5			1	47
	8-MeIQx	0.1			0	6
	8-MeIQx	0.45	190		0	14
	8-MeIQx	1.1	250	10	1	9
	8-MeIQx	1.0	300	6	0	7
	8-MeIQx	0.64			0	35
	8-MeIQx	0.8			1	25
	8-MeIQx	2.95	250	6	1	25
	8-MeIQx	0.0	150	2	0	26
	8-MeIQx	0.0	150	4	0	26
	8-MeIQx	0.15	150	6	0	26
	8-MeIQx	2.7	150	10	0	26
	8-MeIQx	0.1	190	2	0	26
	8-MeIQx	0.25	190	4	0	26
	8-MeIQx	1.3	190	6	0	26
	8-MeIQx	5.1	190	10	0	26
	8-MeIQx	0.0	230	2	0	26
	8-MeIQx	0.4	230	4	0	26
	8-MeIQx	1.1	230	6	0	26
	8-MeIQx	8.0	230	10	0	26
	8-MeIQx	2.2	225	6	1	29
	8-MeIQx	10.8			1	25
	IQ	0.5-20.0	240	5	0	1
	IQ	0.02	250	6	1	6
	IQ	0.5			2	44
	IQ	0.0	192		0	18
	IQ	0.3	275	5	0	39
	IQ	0.3	275	10	0	39
	IQ	1.9	275	15	0	39
	IQ	0.02	300	5.5	1	4
	IQ	0.0	150	4	0	26
	IQ	0.1	150	6	0	26
	IQ	1.5	150	10	0	26
	IQ	0.1	190	2	0	26
	IQ	0.1	190	4	0	26
	IQ	5.3	225	10	1	49

Food type	Mutagen	Amount[1]	Temp[2]	Time[3]	Weight[4]	Ref[5]
	IQ	0.45	190	6	0	26
	IQ	0.82	190	10	0	26
	IQ	0.0	230	2	0	26
	IQ	0.15	. 230	4	0	26
	IQ	0.25	230	6	0	26
	IQ	1.8	230	10	0	26
	IQ	0.0	250	10	1	9
	PhIP	15.0	300	5.5	1	4
	PhIP	67.5	277	6	1	37
	PhIP	1.2	250	10	1	9
	PhIP	5.0			1	25
	PhIP	0.56			1	15
	PhIP	0.0	150	4	0	26
	PhIP	0.25	150	6	0	26
	PhIP	0.9	150	10	0	26
	PhIP	0.15	190	4	0	26
	PhIP	1.9	190	6	0	26
	PhIP	6.0	190	10	0	26
	PhIP	0.55	230	2	0	26
	PhIP	1.35	230	4	0	26
	PhIP	4.1	230	6	0	26
	PhIP	21.5	230	10	0	26
	PhIP	16.4	225	6	1	29
	PhIP	21.8			1	25
	PhIP	1.9-2.6			1	47
	PhIP	31	225	10	1	49
	TMIP	0.5	300	6	0	7
	Trp-P-1	0.19			0	35
	Trp-P-2	0.0	200		0	31
	Trp-P-2	0.21			0	35
BEEF STEAK						
Broiled or Fried	4,8-DiMeIQx	1.3	190	3	1	10
	4,8-DiMeIQx	2.0	190	6.5	1	10
	4,8-DiMeIQx	0.1	225	6	1	33
	8-MeIQx	2.11			0	35
	8-MeIQx	5.1	190	3	1	10
	8-MeIQx	8.3	190	6.5	1	10
	8-MeIQx	0.5	225	6	1	33
	MeIQx	1.7-2.4			1	47
	PhIP	6.8-10.0			1	47
	DiMeIQx	0.1-0.4			1	47

Food type	Mutagen	Amount[1]	Temp[2]	Time[3]	Weight[4]	Ref[6]
	AαC	1.2	0			35
	AαC	3.2	190	3	1	10
	AαC	8.9	190	6.5	1	10
	Glu-P-1	0.0	0			18
	IQ	0.19	0			35
	PhIP	15.7	1			15
	PhIP	23.5	190	3	1	10
	PhIP	48.5	190	6.5	1	10
	PhIP	0.6	225	6	1	33
	Trp-P-1	53.0	0			45
	Trp-P-1	0.21	0			35
	Trp-P-2	0.25	0			35
Grilled	MeIQx	0.2-2.7	160			48
	PhIP	2.5-30.0	160			48
Charbroiled	MeIQx	1.1-1.6	160			48
	PhIP	5.7-15	160			48
Grilled Bonito						
	4,8-DiMeIQx	5.4	220	15	1	22
	8-MeIQx	5.2	220	15	1	22
CHICKEN						
Charbroiled	4,8-DiMeIQx	0.1	1			33
	8-MeIQx	0.3	1			33
Broiled	4,8-DiMeIQx	0.81	1			35
	MeIQx	2.1				44
	8-MeIQx	2.33	1			35
	AαC	0.21	1			35
	PhIP	38.1	1			15
	Trp-P-1	0.12	1			35
	Trp-P2	0.18	1			35
Fried	Trp-P-1	0.0	300	6	0	8
Consomme, Heated						
	4,8-DiMeIQx	0.0	0			33
	8-MeIQx	0.1	0			33
	PhIP	0.0	0			33
EEL						
Fried, Canned	7,8-DiMeIQx	5.3	180	4	1	28
	8-MeIQx	1.1	180	4	1	28

Food type		Mutagen	Amount [1]	Temp[2]	Time[3]	Weight[4]	Ref[6]
Boiled or		4,8-DiMeIQx	0.0	160	2.5	1	17
Smoked		MeIQ	0.0	160	2.5	1	17
		MeIQx	0.6	160	2.5	1	17
		IQ	0.3	160	2.5	1	17
		PhIP	0.0	160	2.5	1	17
FISH							
Smoked Flounder							
		4,8-DiMeIQx	0.6			1	17
		MeIQ	0.3			1	17
		MeIQx	0.0-2.9			1	17
		IQ	0.7			1	17
		PhIP	0.0			1	17
Fried Herring							
		4,8-DiMeIQx	0.3			1	17
		MeIQ	0.1			1	17
		MeIQx	0.6			1	17
		IQ	0.2			1	17
		PhIP	0.0			1	17
Fried Pollack							
		4-MeIQ	0.03	260	8	1	46
		4,8-DiMeIQx	0.1	260	8	1	46
		8-MeIQx	6.44	260	8	1	46
		IQ	0.16	260	8	1	46
		PhIP	69.2	260	8	1	46
Baked Salmon							
		8-MeIQx	0.0	200	20	1	11
		8-MeIQx	4.6	200	30	1	11
		8-MeIQx	3.1	200	40	1	11
		AαC	0.0	200	20	1	11
		AαC	0.0	200	30	1	11
		AαC	0.0	200	40	1	11
		PhIP	0.0	200	20	1	11
		PhIP	18.0	200	30	1	11
		PhIP	5.9	200	40	1	11
Broiled Salmon	*Flesh*	4-MeIQ	0.6-2.8			1	44
	Skin	4-MeIQ	1.1-1.7			1	44
		4-MeIQ	0.1-0.9			1	3
		MeIQ	1.4-5.0			1	11
	Flesh	IQ	0.3-1.8			1	44

Food type	Mutagen	Amount [1]	Temp[2]	Time[3]	Weight[4]	Ref[6]
Broiled Salmon Skin	IQ	1.1-1.7			1	44
Broiled Salmon	IQ	0.2-0.4			1	3
	PhIP	1.7-23.0			1	11
Charbroiled Salmon	8-MeIQx	0.0	270	4	1	11
	8-MeIQx	0.0	270	6	1	11
	8-MeIQx	0.0	270	9	1	11
	8-MeIQx	0.0	270	12	1	11
	AαC	2.8	270	4	1	11
	AαC	6.9	270	6	1	11
	AαC	73.0	270	9	1	11
	AαC	109.0	270	12	1	11
	PhIP	2.0	270	4	1	11
	PhIP	6.2	270	6	1	11
	PhIP	69.0	270	9	1	11
	PhIP	73.0	270	12	1	11
Cooked Salmon	4,8-DiMeIQx	0.2	150	9	1	17
	MeIQ	1.0-1.6	150	9	1	17
	MeIQx	0.6	150	9	1	17
	IQ	0.6	150	9	1	17
	PhIP	2.7-3.3	150	9	1	17
Fried Salmon	8-MeIQx	1.4	200	3	1	11
	8-MeIQx	5.0	200	6	1	11
	8-MeIQx	4.7	200	9	1	11
	8-MeIQx	3.7	200	12	1	11
	AαC	0.0	200	3	1	11
	AαC	4.6	200	6	1	11
	AαC	8.0	200	9	1	11
	AαC	9.0	200	12	1	11
	PhIP	1.7	200	3	1	11
	PhIP	23.0	200	6	1	11
	PhIP	14.0	200	9	1	11
	PhIP	17.0	200	12	1	11
Smoked Salmon	4,8-DiMeIQx	0.0			0	17
	MeIQ	0.0			0	17
	MeIQx	1.2-1.4			0	17
	IQ	0.3			0	17
	PhIP	0.0			0	17
Broiled Sardine						
	4-MeIQ	16.6			1	45

Food type	Mutagen	Amount 1	Temp[2]	Time[3]	Weight[4]	Ref[6]
Broiled Sardine	4-MeIQ	20.0			0	20
	8-MeIQx	0.0			2	45
	Glu-P-1	0.0			1	45
	IQ	20.0			0	19
	IQ	4.9			1	44
	IQ	20.0			0	20
	Phe-P-1	8.6			1	45
	Trp-P-1	13.3			1	45
	Trp-P-2	13.1			1	45
UNSPECIFIED						
Fried	Trp-P-2	0.0	200		1	31
Heated	4,8-DiMeIQx	5.4			1	23
	MeIQx	5.2			1	23
Smoked, Dried	4,8-DiMeIQx	0.08			1	21
	MeIQx	0.8			1	21
LAMB						
Broiled	4,8-DiMeIQx	0.67			1	35
	8-MeIQx	1.01			1	35
	AαC	2.5			1	35
	AMαC	0.19			1	35
	PhIP	42.5			1	15
	Trp-P-2	0.15			1	35
Fried Meatballs						
	4,8-DiMeIQx	0.2			1	17
	MeIQ	0.3			1	17
	MeIQx	0.7			1	17
	IQ	0.2			1	17
	PhIP	0.6			1	17
Boiled Meat Extract						
	4,8-DiMeIQx	2.9-3.6			1	34
	8-MeIQx	6.2-28.3			1	34
	IQ	1.9-4.8			1	34
PORK						
Charbroiled	4,8-DiMeIQx	0.1			0	33
	8-MeIQx	0.4			0	33
	PhIP	4.2			0	33
Fried	Trp-P-1	0.0	300	6	0	8

Food type	Mutagen	Amount [1]	Temp[2]	Time[3]	Weight[4]	Ref[6]
Fried Bacon						
	MeIQx	0.9-18.0		12-16	1	12
	4,8-DiMeIQx	0.0-1.		12-16	1	12
	PhIP	0.0-53.0		12-16	1	12
	MeIQx	0.4-4.3	160		1	48
	PhIP	0.7-4.8	160		1	48
Moderate	4,8-DiMeIQx	1.7-5.1	150	2.5	1	17
Well-done	4,8-DiMeIQx	1.0	150	5	1	17
Well-done	MeIQ	1.4-2.0	150	5	1	17
Moderate	MeIQx	0.0-5.8	150	2.5	1	17
Well-done	MeIQx	1.4-3.6	150	5	1	17
Moderate	IQ	2.3-5.3	150	2.5	1	17
Well-done	IQ	9.5-11.5	.150	5	1	17
Moderate	PhIP	0.2	150	2.5	1	17
Well-done	PhIP	1.0	150	5	1	17
Broiled Bacon	MeIQx	1.5-4.0	160		1	48
	PhIP	1.4-30.3	160		1	48
Microwaved Bacon	MeIQx	0.4-1.5			1	47
	PhIP	3.1			1	47
Fried Bacon Fatty						
	4,8-DiMeIQx	0.3	225	6	1	33
	8-MeIQx	1.2	225	6	1	33
	PhIP	2.7	225	6	1	33
Fried Bacon Lean						
	4,8-DiMeIQx	0.2	225	6	1	33
	8-MeIQ	0.9	225	6	1	33
	PhIP	1.6	225	6	1	33
Fried Ground Pork						
	4,8-DiMeIQx	0.6	250	5	0	42
	4,8-DiMeIQx	0.24	180		1	2
	4-MeIQ	0.02	250	5	0	42
	4-MeIQx	0.1	250	5	0	42
	4-MeIQx	1.4	250	5	0	42
	4-MeIQx	0.4	180		1	2
	IQ	0.04	250	5	0	42
	IQ	0.01	180		1	2
	PhIP	4.5	250	5	0	42
	PhIP	1.7	180		1	2
	PhIP	0.0			0	13

Food type	Mutagen	Amount[1]	Temp[2]	Time[3]	Weight[4]	Ref[5]
Fried Ground Pork	4,8-DiMeIQx	0.9	250		1	2
	8-MeIQx	1.5	250		1	2
	IQ	0.04	250		1	2
	PhIP	10.0	250		1	2
Fried Pork Sausage	4,8-DiMeIQx	0.2	160	6	1	17
	MeIQ	0.2	160	6	1	17
	MeIQx	0.7	160	6	1	17
	IQ	0.1	160	6	1	17
	PhIP	0.1	160	6	1	17
	Trp-P-1	0.0	300	6	0	8

AαC = 2-amino-9*H*-pyrido[2,3-*b*]indole; AMαC = 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole; 4-OH-PhIP = 2-amino-l-methyl-6-(4-hydroxyphenyl)imidazo[4,5-*b*]pyridine; 4-CH_2OH-8-Mex = 2-amino-4-hydroxy-methyl-3,8-dimethylimdazo[4,5-*f*] quinoxaline; Trp-P-1 = 3-amino-l,4-dimethyl-5*H*-pyrido[4,3-*b*]indole; Trp-P-2 = 3-amino- l-methyl-5*H*-pyrido[4,3-*b*]indole; Glu-P-1 = 2-amino-6-methyldipyrido[l,2-a:3',2'-*d*]imidazole; Glu-P-2 = 2-aminodipyrido[l,2-a:3',2'-*d*] imidazole.

[1] Amount of mutagen formed (ng/g)
[2] Temperature of frying (°C)
[3] Cooking time (minutes per side), except for sausage (total frying time)
[4] Basis for heterocyclic aromatic amine concentration.
0 = cooked weight of food, l = uncooked weight, 2 = unspecified
[5] References:

1- Barnes et al., 1983
2- Dragsted, 1992
3- Edmonds et al., 1986
4- Felton et al., 1986a
5- Felton et al., 1992
6- Felton et al., 1984
7- Felton et al., 1986b
8- Felton et al., 1997
9- Gross et al., 1989
10- Gross, 1990
11- Gross and Grűter, 1992
12-Gross et al., 1993
13- Gry et al., 1986
14- Hargraves and Pariza, 1983
15- Hayatsu et al., 1991
16- Jackson et al., 1994

17- Johansson and Jägerstad, 1994
18- Kasai et al., 1981b
19- Kasai et al., 198la
20- Kasai et al., 1980
21- Kato et al., 1986
22- Kikugawa et al., 1986
23- Kikugawa and Kato, 1987
24- Kim et al., 1994
25- Knize et al., 1990
26- Knize et al., 1994
27- Kurosaka et al., 1992
28- Lee and Tsai, 1991
29- Lynch et al., 1992
31- Murray et al., 1987
32- Murray et al., 1988
33- Murray et al., 1993
34- Schuirmann and Eichner, 1991
35- Sugimura et al., 1988
36- Taylor et al., 1985
37- Thiebaud et al., 1994
38- Turesky et al., 1983
39- Turesky et al., 1988
40- Turesky et al., 1989
41- Takahashi et al., 1985
42- Vahl et al., 1988
43- Wakabayashi et al., 1986
44- Yamaizumi et al., 1986
45- Yamaizumi et al., 1980
46- Zhang et al., 1988
47- Sinha et al., 1998
48- Knize et al., 1998
49- Balogh et al., 2000

identified in fried ground beef (Barnes et al., 1983; Felton et al., 1984; Felton et al., 1986b; Turesky et al., 1983), boiled beef extract (Gross et al., 1989; Hargraves and Pariza et al., 1983), charbroiled ground beef (Johansson and Jägerstad, 1994), broiled ground beef (Yamaizumi et al., 1986), broiled or fried steak (Sugimura et al., 1988), smoked, fried, and broiled fish (Johansson and Jägerstad, 1994; Yamaizumi et al., 1986; Zhang et al., 1988), fried meat balls (Johansson and Jägerstad, 1994), and fried ground pork (Vahl et al., 1988). The presence of IQ in fried ground beef has been reported at

various concentrations ranging from 0 to 20 ng/g (Barnes et al., 1983; Felton et al., 1984; Turesky et al., 1988). However, MeIQ was found in relatively smaller concentrations (Felton et al., 1986a; Gross et al., 1993; Yamaizumi et al., 1986).

Quinoxalines

The quinoxaline, MeIQx, was first isolated from fried ground beef, followed by 2-amino-3,7,8-trimethyl imidazo[4,5-*f*]-quinoxaline (7,8-DiMeIQx) (Negishi et al., 1984), and 4,8-DiMeIQx (Grivas et al., 1985). MeIQx was identified when a model system containing creatinine, glucose and glycine was heated for two hours at 128 °C. Overvik et al. (1989) showed that model systems containing creatinine, threonine, serine, or alanine produced MeIQx. 7,8-DiMeIQx has been found in boiled beef extract (Turesky et al., 1988), fried ground beef (Felton et al., 1986b; Turesky et al., 1988), and roasted, fried, and canned eel (Lee and Tsai, 1991). 4,8-DiMeIQx has been isolated from fried, charbroiled, and grilled ground beef (Felton et al., 1986a, 1992; Grivas et al., 1985; Murray et al., 1988; Sugimura et al., 1988; Turesky et al., 1988), broiled and roasted beef extracts (Gross et al., 1989, 1990; Hargraves and Pariza, 1983; Murray et al., 1993; Turesky et al., 1988), grilled bonito (Kikugawa et al., 1986), charbroiled and broiled chicken (Murray et al., 1993; Sugimura et al., 1988), boiled, smoked, and fried sausage (Johansson and Jägerstad, 1994), smoked and fried fishes (Johansson and Jägerstad, 1994; Zhang et al., 1988), broiled lamb (Sugimura et al., 1988), fried meatballs (Johansson and Jägerstad, 1994), charbroiled pork (Murray et al., 1993), and fried bacon (Murray et al., 1993).

Pyridines

The most abundant HAA in cooked meat is PhIP (Felton et al., 1986a; Gry et al., 1986; Hayatsu et al., 1991; Skog et al., 1997; Vahl et al., 1988). It was first isolated from the crust of fried ground beef (Felton et al., 1986b). Phenylalanine and glucose have been reported to be its precursors (Felton and Knize, 1990; Manabe et al., 1992; Overvik et al., 1989; Shioya et al., 1987; Skog and Jägerstad, 1991). There is some evidence that leucine may also be involved in PhiP formation (Overvik et al., 1989). Skog and Jägerstad (1990, 1991) demonstrated the effect of glucose on the formation of PhIP by heating a mixture of creatine, phenylalanine and glucose in diethylene glycol and water. In addition, MeIQx and 4,8-DiMeIQx were also formed. Heating the model system without glucose produced PhIP as a single mutagen, but in a relatively smaller amount. Recently, Arvidsson et al. (1999) reported that PhIP and other polar HAAs were formed in heated mixtures containing creatinine, glucose and amino acids in proportions similar to those in bovine meat but at higher concentrations.

Furopyridines

The forementioned HAAs account for 30-75% of the total mutagenicity in various cooked foods (Stavric, 1994). Other less-well characterized mutagenic HAAs are present in cooked meat products (Felton et al., 1984, 1986b; Skog et al., 1998; Zhang et al., 1988). A methylimidazo-furopyridine (MeIFP) containing oxygen, with a molecular weight of 202, has been identified in fried minced beef containing milk and creatine (Knize el al., 1990). These investigators suggested that this mutagen is related to the food mutagen with a molecular weight of 216, an amino-

dimethylimidazofuropyridine. Such HAAs have been isolated also from fried ground beef, pork, and fried meat emulsion (Becher et al., 1988; Felton et al., 1986a; Gry et al., 1986; Skog et al., 1998).

Non-polar heterocyclic aromatic amines

Mutagenic and/or carcinogenic HAAs have also been classified as polar and non-polar compounds. To obtain a better estimation of the total concentration of HAAs in cooked foods, analyses have been extended to include non-polar HAAs such as 3-amino-l,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), 2-amino-6-methyl-pyrido[l,2-a:3',2'-*d*]imidazole (Glu-P-1), 2-amino dipyridol [l,2-a:3',2'-*d*]imidazole (Glu-P-2), the amino-α-carbolines, 2-amino-9*H*-pyrido[2,3-*b*]indole (AαC) and 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole, (MeAαC) and the β-carbolines, l-methyl-9*H*-pyrido[3,4-*b*]indole (harman) and 9*H*-pyrido[3,4-*b*]indole (norharman). Trp-P-1 and Trp-P-2 have been shown to induce liver tumors in mice and rats. The latter has also been implicated in urinary bladder cancer (Takahashi et al., 1993). Chen and Meng (1999) reported that norharman, harman, AαC, and MeAαC were produced in a model system containing phenylalanine, glucose, and creatinine, when heated at 150 or 200°C. The amino-α-carbolines, AαC and MeAαC, were also formed in model systems containing glucose and creatinine at 150 or 200°C. Abdulkarim and Smith (1998) demonstrated the presence of norharman, harman, and Trp-p-2 on the surfaces of processed meat samples, including fresh pork sausage, bratwurst, Italian sausage, and smoked sausage. They also reported high concentrations of norharman (30.0 ng/g), harman (28.6 ng/g), and Trp-p-2 (1.59 ng/g)

in beef steak that was grilled at 240°C for 14 min. Non-polar HAAs have not received as much attention as the polar HAAs because they were thought to be formed exclusively under extreme cooking conditions and were not believed to be found in the Western diet (Lansen et al; 1990).

Other heterocyclic aromatic amines

With the improvements in isolation and identification methodologies, several other HAAs have been identified. TMIP (2-amino-l,5,6-trimethyl- imidazopyridine) and DMIP (2-amino-l,6-dimethyl- imidazopyridine) have been isolated from fried meat products (Becher el al., 1988; Felton el al., 1984). In addition, a number of oxygen-containing HAAs has been identified. A methylimidazo-furopyridine was identified in fried minced beef containing milk and creatine (Knize et al., 1990). Knize et al. (1991) reported the formation of 2,6-diamino-3,4-dimethyl-7-oxo-pyrano[4,3-*g*]benzimidazole in a dry-heated mixture of creatine, glutamic acid and glucose. 4,7,8-TriMeIQx (2-amino-3,4,7,8- tetramethylimidazo[4,5-*f*]quinoxaline) was identified in a model system containing alanine, threonine, creatinine and glucose (Skog el al., 1992). Several HAAs, identified as 4'-OH-PhIP (2-amino-l-methyl-6-(4-hydroxyphenyl)-imidazo[4,5-*b*] pyridine), 4-CH_2OH-8-MeIQx (2-amino-4-hydroxy-methyl-3,8-dimethylimidazo-[4,5-*f*-quinoxaline) and 7,9-DiMeIQx (2-amino-l,7,9-trimethylimidazo [4,5-*g*]-quinoxaline), were isolated in broiled, fried beef, or bacteriological grade beef extracts (Kurosaka el al., 1992; Reistad el al., 1997; Wakabayashi el al., 1995). The 4'-OH-PhIP was produced on heating a liquid model system containing tyrosine, creatine and glucose (Wakabayashi el al., 1995). 4-

CH_2OH-8- MeIQx (Nukaya el al., 1994; Wakabayashi el al., 1995) and 7,9-DiMeIgQx (Wakabayashi el al., 1995) were identified on heating creatine, threonine and glucose in a model system. The identification of these relatively new HAAs adds to our overall knowledge of HAA formation in foods and illustrates the importance for continuing research in this area.

Mutagenicity of Heterocyclic Aromatic Amines

Several HAAs have been shown to be extremely mutagenic when subjected to the Ames *S. typhimurium* mutagenicity test (Felton, 1997; Felton et al., 1986a; Sugimura, 1988). They also exhibit moderate mutagenicity in mammalian cell cultures and cause chromosomal change in mice (Felton and Knize, 1990). Both IQ and MeIQ exhibit mutagenicity in the Ames *S. typhimurium* mutagenicity test with strains of TA 98, TA 100, TA 1537, TA 1538, and TA 1978 (Kasai et al., 1981a). In contrast to IQ, MeIQx and 4,8-DiMeIQx, PhIP exhibits relatively weak mutagenic activity in the *S. typhimurium* TA 98, TA 100, and TA 1538. However, PhIP was more mutagenic in cultured mammalian cells (Alink et al., 1988; Felton et al., 1986b).

HAAs are metabolically activated by cytochrome P450. Initially, 2-hydroxyamino-3-methyl-3H-imidazo[4,5-*f*]quinoline (N-hydroxy-IQ) is produced as an metabolite from the N-hydroxylation of IQ by cytochrome P450 monooxygenase. This is followed by esterification to an acetyl or sulfate moiety (Okamoto et al., 1981; Paterson and Chipman, 1987; Saito et al., 1985; Snyderwine et al., 1987). Human liver cytochrome P450 isoforms, P4501A2, P450HFLa, and P4503A4, metabolically activate IQ in fetal livers (Kitada et al., 1990, 1991). Aoyama et al. (1990) and Butler et al.

(1989) reported that cytochrome P4501A2 is also the enzyme for the activation of MeIQ, MeIQx, and 4,8-DiMeIQx. Mutagenicity varies widely among individual HAAs, but can be as high as 661,000 revertants/μg when evaluated using *S. typhimurium* TA98. Aflatoxin B1, a well-documented carcinogen, produces only 6,000 revertants/μg under similar conditions (Table 2). Not all mutagens are carcinogens, however, and carcinogenicity must be determined with animal or cell culture studies. Rodent and primate assays have shown many HAAs to be multisite carcinogens, including IQ, MeIQ, MeIQx, and PhIP, as well as many of the nonpolar HAAs such as Trp-P-1, Trp-P-2, Glu-P-1, Glu-P-2, AαC, and MeAαC (Ohgaki et al., 1991).

Several studies have shown that IQ, MeIQ, and MeIQx can produce tumors in the liver, lung, forestomach, small and large intestines, zymbal gland, skin, colon, and the lymph system of mice and rats (Stavric, 1994). Mutation of *Ha-ras*, *Ki-ras*, and *p53* have been observed in zymbal gland tumors in F344 rats induced by IQ, MeIQ, and MEIQx (Esumi et al., 1989; Ito et al., 1991; Takayama, 1984). PhIP induced mammary gland carcinomas and colon cancer in rodents (Stavric, 1994). A specific mutation of the Apc gene-deletion of a guanine base at 5'-GGGA-3' site was observed by PhIP-induced colon cancers in rat (Ohgaki et al., 1984, 1986, 1987; Wakabayashi and Sugimura, 1998). Urinary bladder cancers developed by TRP-P-2 in rats and MeAαC caused atrophy in the salivary glands and pancreas of rats (Wakabayashi and Sugimura, 1998).

Formation of Heterocyclic Aromatic Amine in Foods

Table 2. Mutagenicity of heterocyclic aromatic amines and typical carcinogens as determined by the Ames *Salmonella typhimurium* assay (Sugimura and Sato, 1982; Sugimura et al., 1988).

	Revertants/ug	
Compound	**TA98**	**TA100**
IQ	433,000	7,000
MeIQ	661,000	30,000
IQx	75,000	1,500
MeIQx	145,000	14,000
4,8-DiMeIQx	183,000	8,000
7,8-DiMeIQx	163,000	9,900
PhIP	1,800	120
Trp-P-1	39,000	1,700
Trp-P-2	104,200	1,800
Glu-P-1	49,000	3,200
Glu-P-2	1,900	1,200
Orn-P-1	56,800	
AαC	300	20
MeαAC	200	120
Aflatoxin B1	6,000	28,000
AF-2	6,500	42,000
4-Nitroquinolin-1-oxide	970	9,900
Benzo[*a*]pyrene	320	660
N-Methyl-N'nitro-N-nitrosoguanidine	0.00	870
N-Nitrosodiethylamiine	0.02	0.15
N-Nitrosodimethylamine	0.00	0.23

Reactants

The mode of formation of HAAs in cooked meats has not been fully clarified. Yoshida and Okamoto (1980) reported that dry heating creatine with either glucose, fatty acids or various amino acids at 100-200°C produced high mutagenic activity, and suggested that these reactants were possible HAA precursors. Jägerstad et al. (1983a) suggested that creatine, free amino acids, and hexose, were precursors of the imidazoquinolines and imidazoquinoxalines. Chicken and beef contain the same HAAs in similar proportions as does fried ground fish, although in smaller amounts, thus suggesting that HAAs in cooked muscle foods all have similar precursors (Felton and Knize, 1991).

Supporting evidence for creatine/creatinine involvement in HAA formation is the low mutagenic activity in cooked food products lacking in creatine (Felton and Knize, 1990; Reutersward et al., 1987b). Furthermore, Jägerstad et al. (1983a) demonstrated a significant increase in mutagenic activity in beef when creatine was spread over the surface before frying. The lack of mutagenic activity in cooked shrimp can also be explained by the low levels of creatinine (Bjeldanes et al., 1982).

Free amino acids play an important role in HAA formation. Overvik et al. (1989) reported that mutagenic activity was detected when amino acids were heated with creatine at 200 °C. Data summarized in Table 3 indicate that amino acid can produce various HAAs when heated with sugar and creatinine.

Sugar involvement in HAA formation was demonstrated by Negishi et al. (1984),

Table 3. Heterocyclic aromatic amines produced in model systems

Compound	Yield*	Amino Acid	Sugar	Heating Conditions	Reference
IQ	0.4	pro		Dry	Yoshida et al. (1984)
	1.0	gly	Fru	DEG-Water	Grivas et al. (1986)
	3.0	phe	-	Dry	Felton and Knize (1990)
	13.5	phe	glu	Dry	Felton and Knize (1990)
	3.7	ser	-	Dry	Knize et al., (1998)
MeIQ	nd	ala	fru	DEG-Water	Grivas et al. (1985)
IQx	2.7	ser	-	Dry	Knize et al. (1998)
	nd	thr	glu	Water	Skog and Jägerstad (1993)
	0.025	phe	glu	Water	Chen and Meng (1999)
	65	meat	meat	Water	Skog et al. (2000)
MeIQx	4.4	gly	glu	DEG-Water	Jägerstad et al. (1983b)
	0.9	ala	glu	DEG-Water	Muramatsu and Matsushima (1985)
	1.8	ala	rib	DEG-Water	Muramatsu and Matsushima (1985)
	4.2	lys	rib	DEG-Water	Muramatsu and Matsushima (1985)
	nd	thr	glu	DEG-Water	Negishi et al. (1984)
	6-7	gly	fru	DEG-Water	Grivas et al. (1986)
	nd	ser	-	Dry	Overvik et al. (1989)
	nd	ala	-	Dry	Overvik et al. (1989)
	nd	tyr	-	Dry	Overvik et al. (1989)
	4	elv	glu	DEG-Water	Skog and Jägerstad (1990)
	nd	phe	glu	DEG-Water	Skog and Jägerstad (1991)
	10	ala, thr	glu	DEG-Water	Skog et al. (1992)
	8.8-17.9	gly	glu	Water	Johansson and Jägerstad (1993)
	7-10	gly	glu	Water	Skog and Jägerstad (1993)
	9	thr	glu	Water	Skog and Jägerstad (1993)
	nd	gly	glu	Water	Johansson and Jägerstad (1993)
	110	meat	meat	Water	Skog et al. (2000)
4,8-DiMeIQx	nd	thr	glu	DEG-Water	Negishi et al. (1984)
	1.9-2.6	ala	fru	DEG-Water	Grivas et al. (1985)
	4.2	ala	glu	DEG-Water	Muramatsu and Matsushima (1985)
	1.5	ala	rib	DEG-Water	Muramatsu and Matsushima (1985)
	26.1	lys	rib	DEG-Water	Muramatsu and Matsushima (1985)
	nd	gly	glu	DEG-Water	Skog and Jägerstad (1990)
	nd	phe	glu	DEG-Water	Skog and Jägerstad (1991)
	36	ala, thr	glu	DEG-Water	Skog et al. (1992)
	30	thr	glu	Water	Skog and Jägerstad (1993)
	nd	gly	glu	Water	Johansson et al. (1993)
7,8-DiMeIQx	1.1	gly	glu	DEG-Water	Negishi et al. (1984)
	Nd	gly	glu	DEG-Water	Skog and Jägerstad (1990)

	Nd	gly	Glu	Water	Johansson and Jägerstad (1993)
4,7,8-TriMeIQx	6	ala, thr	Glu	DEG-Water	Skog et al. (1992)
PhIP	3.6	phe	glu	DEG-Water	Shioya et al. (1987)
	735	phe	-	Dry	Felton and Knize (1990)
	560	phe	glu	Dry	Felton and Knize (1990)
	Nd	phe	-	Dry	Overvik et al. (1989)
	Nd	leu	-	Dry	Overvik et al. (1989)
	20.9	phe	glyu	DEG-Water	Skog and Jägerstad (1991)
	6.4	phe	-	DEG-Water	Skog and Jägerstad (1991)
	0.06	phe	glu	DEG-Water	Manabe et al. (1992)
	14	meat	meat	Water	Skog et al. (2000)
Norharman	0.035	phe	glu	Water	Chen and Meng (1999)
Harman	0.035	phe	glu	Water	Chen and Meng (1999)

As adapted from Skog, 1993.
*Yield is in nmol/mmol creatin(in)e.
Dry = dry heating at 180 or 200°C for 1 hour.
DEG-Water = reflux boiling in diethylene glycol/water (5:1) or 14% water.
Water = heated in water in closed metal tubes at 180°C for up to 30 minutes.
Amino acids: pro = proline, gly = glycine, phe = phenylalanine, ser = serine, ala = alanine, thr = threonine, lys = lysine, tyr = tyrosine, leu = leucine.
Sugars: fru = fructose, glu = glucose, rib = ribose.
nd = not determined

but its role as a precursor still remains unclear. Many studies have addressed the impact of various sugars on HAA formation in model systems containing creatine/creatinine, and amino acids (Manabe et al., 1992; Muramatsu and Matsushima, 1985; Skog and Jägerstad, 1990, 1991). The incorporation of carbon atoms from ^{14}C-labelled glucose into IQx, MeIQx and 4,8-DiMeIQx under model system conditions confirmed that glucose is an important precursor for HAA formation (Skog and Jägerstad, 1993).

Effect of cooking time and temperature on HAA formation

A number of investigators have demonstrated that HAA formation increases with increasing temperature of cooking (Balogh et al., 2000; Bjeldanes et al., 1983; Chen, 1988; Hatch et al., 1982; Knize et al., 1994; Sinha et al., 1998; Spingarn and

Weisburger, 1979). Cooking methods that employ higher heating temperatures generally induce greater HAA formation than do lower temperature methods (Sinha et al., 1998). Several investigators also have observed that there is a rapid increase in HAA formation with increasing cooking time (Balogh et al., 2000; Chen, 1988; Knize et al., 1994; Sinha et al., 1998). However, there is a lag period of 0 to 2 min during the frying of ground beef patties when no HAA formation is observed. This is the time required for the meat patty crust surface to reach temperature of 100°C and above (Knize et al., 1994).

Recent work by Balogh et al. (2000) confirmed the increase in HAA formation in ground beef patties with increasing time and/or temperature of frying. There was a pronounced increase in the formation of HAAs when the temperature was raised from 175°C to 200°C and 225°C. Concentrations of total HAAs formed at 225°C were six times greater than corresponding quantities formed at 175°C. Concentrations of total HAAs also increased 2-5 times when cooking times were increased from 6 min to 10 min.

The most abundant HAA in fried ground beef is PhIP. Balogh et al. (2000) demonstrated that concentrations of PhIP in the beef patties fried at 225°C for 10 min were approximately 10 times greater than those of MeIQ. Arvidsson et al. (1999) reported that a longer time and a higher cooking temperature are necessary to produce PhIP in model systems containing creatinine, glucose, and amino acids because IQx, MeIQx, and DiMeIQx have lower activation energies of formation than PhIP.

Chemistry of HAA formation

A possible route of HAA formation is through intermediates of the Maillard or non-enzymatic browning reaction (Powrie et al., 1981; Shibamoto et al., 1981; Spingarn and Garvie, 1979; Wei et al., 1981). The Maillard reaction takes place in food through a series of reactions between reducing sugars such as glucose and fructose, and compounds possessing available amino groups such as amino acids, peptides, and proteins. The combining of an available amino group and a reducing sugar produces a glycosylamine which undergoes an Amadori rearrangement to produce a 1-amino-2-keto sugar (Figure 2). This intermediate may then be broken down into 2- and 3-carbon fragments by two pathways (3-deoxyhexosone and methyl α-dicarbonyl routes), leading to the formation of a variety of compounds such as aldehydes, ketones and melanoidin pigments. Pyrazine and pyridines can be produced from the interaction of the α-dicarbonyls from the Maillard reaction with amino acids, the so-called Strecker degradation (Rizzi, 1994). Aldehydes, furanones, sulfur-containing heterocyclic compounds, pyridines, pyrazines, and pyrroles are volatile compounds produced via the Maillard reaction which contribute to the flavor and aroma of cooked foods. Melanoidines are color compounds formed during the Maillard reaction which contribute to brown color formation in breads and cooked meats (Rizzi, 1994).

A mechanism for the early stages of the Maillard reaction identifying the Amadori rearrangement as a key step was proposed by Hodge (1953). This was subsequently questioned by Namiki and Hayashi (1981). They reported the formation of the N,N'-disubstituted pyrazine cation by early carbon fragmentation prior to the Amadori product. They demonstrated that radical products are formed by the

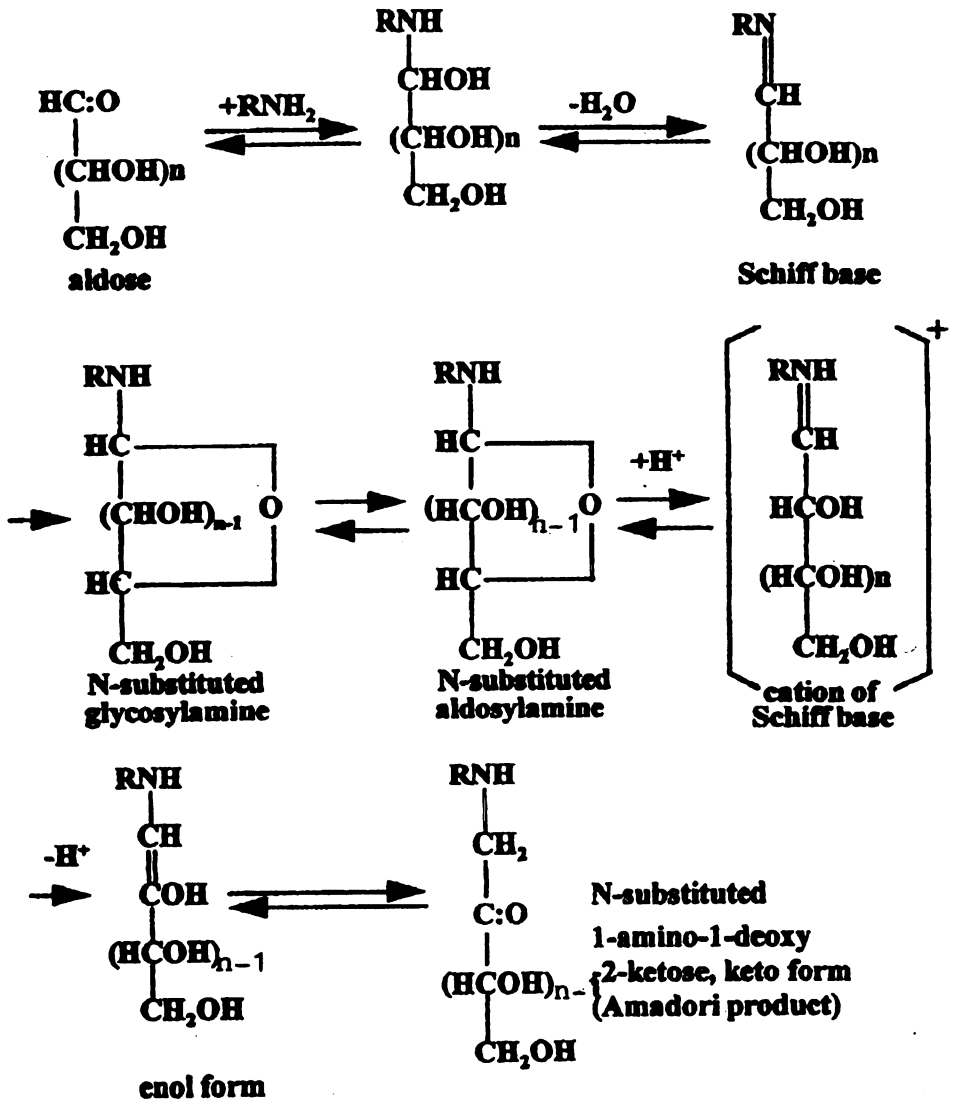


Figure 2. Initial steps of the Maillard reaction (Hodge, 1953).

condensation of two molecules of the two-carbon enaminol compounds which might be formed either directly from Schiff's base products or indirectly through the reaction of glycoaldehyde with amino compounds (Figure 3). They also reported that C2 and C3 fragments were produced prior to the Amadori rearrangement by a reverse-aldol reaction of the glycosylamine leading to the formation of a glycolaldehyde alkylimine. The pyridine radical would result from the interaction of glyoxal monoalkylimine with glyoxal. This compound can then be oxidized to form a glyoxal monoalkylimine, which produced less free radical and reacted more slowly than the glycoaldehyde system (Namiki and Hayashi, 1981, 1983). Glycoaldehyde is very effective in facilitating rapid and extensive radical formation compared to glyoxal.

A possible mechanism of HAA formation through the Maillard reaction pathway was proposed by Jägerstad et al. (1983b). They assumed that pyridines and pyrazines, formed via the Maillard reaction, react with an aldehyde to form the quinoline or quinoxaline moiety which is central to the structure of HAAs. Creatine is dehydrated by heat and cyclized to creatinine which reacts with the aldehyde to form an IQ-or IQx-type HAA (Figure 4). This theory was confirmed in a model system study in which creatinine, glycine or alanine, and glucose, dissolved in diethylene glycol containing 14% water, were boiled under reflux at 130 °C for 2 hr.

The mixture showed high mutagenic activity, whereas heating the reactants in combinations of two produced only weak, if any, mutagenic activity. The addition of synthetic pyridines or pyrazines to the reaction mixture increased the mutagenic activity by 50% (Jägerstad et al., 1983b). Grivas et al. (1985) utilized a similar model system, employing fructose as the sugar and glycine as the amino acid. They isolated MeIQx

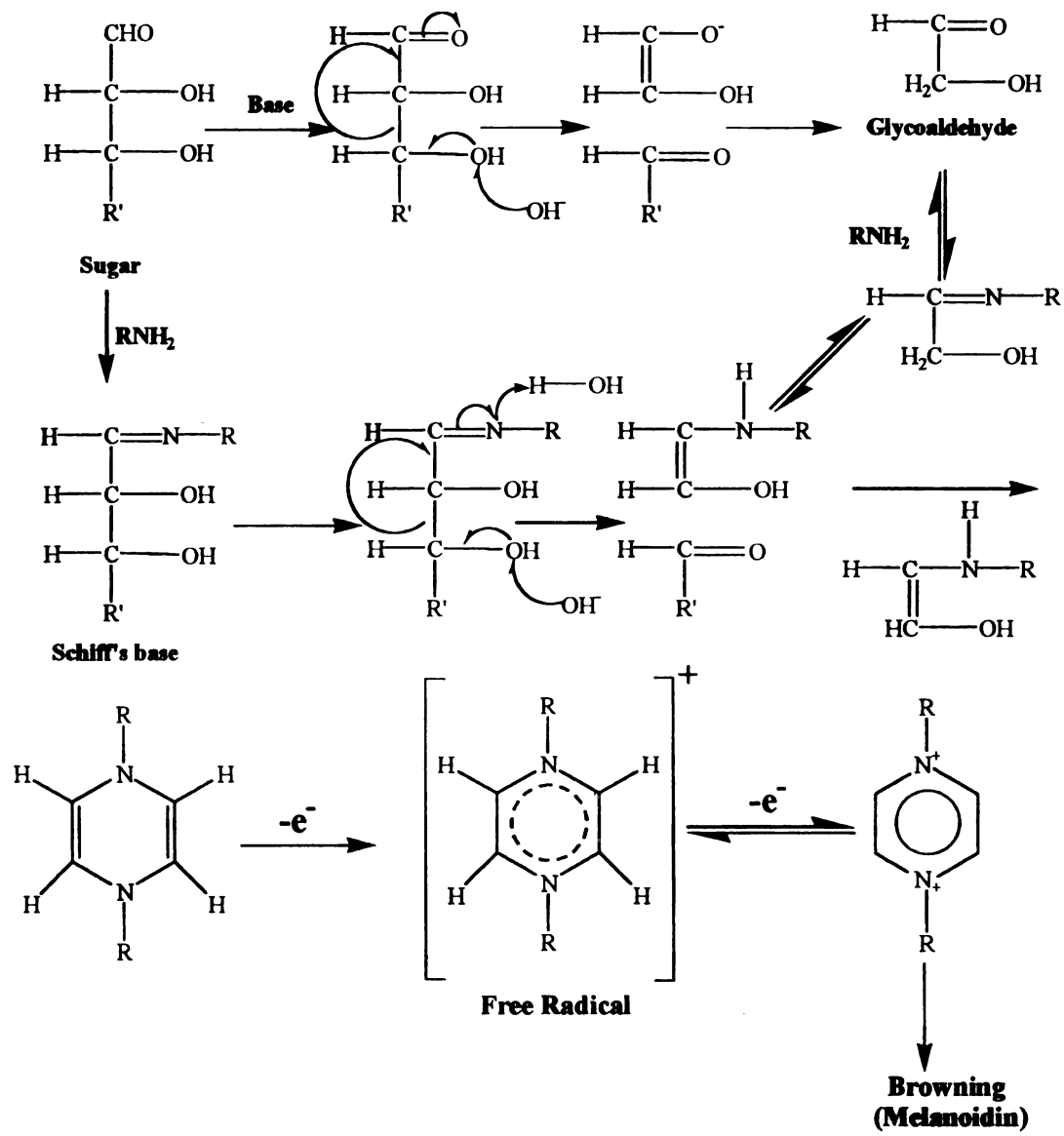


Figure 3. A suggested pathway of browning in the Maillard reaction through a free radical (Namiki and Hayashi, 1981).

and IQ, thus offerred further confirmation of the precursors needed to form HAAs. Support for the condensation of aldehydes with creatinine was proposed by Jones and Weisburger (1988) who reported the formation of different IQ-like mutagenic products through reactions between creatinine and different aldehydes. This model involved the condensation of two molecules of acetaldehyde with creatinine in one step to form IQx-type compounds. They found no mutagenic activity in model systems containing 2-vinylpyrazine and creatinine, an observation that may indicate that aldehydes are necessary for HAA formation.

In a later study, Nyhammer (1986) proposed that HAAs were formed by an aldol-type condensation between an aldehyde and a pyridine or pyrazine molecule, followed by the cyclic addition of creatinine to yield either an imidazoquinoline or an imidazoquinoxaline. Namiki and Hayashi (1975, 1980) proposed that HAA formation may involve a free radical process because free radicals have been shown to occur in the Maillard reaction, both prior to and following the Amadori rearrangement. Milic et al. (1993) provided further support with electron spin resonance studies of HAA formation which confirmed pyrazine/pyridine free radical cation involvement. The pyrazine and pyrazine free radicals were suggested to be precursors of HAAs.

Reduction of Heterocyclic Aromatic Amine Formation in Foods

Many studies have shown that compounds such as sugars and other carbohydrates, soy protein concentrates and defatted glandless cottonseed flour, antioxidants, tea phenolics, and several compounds from fruits and vegetables inhibit

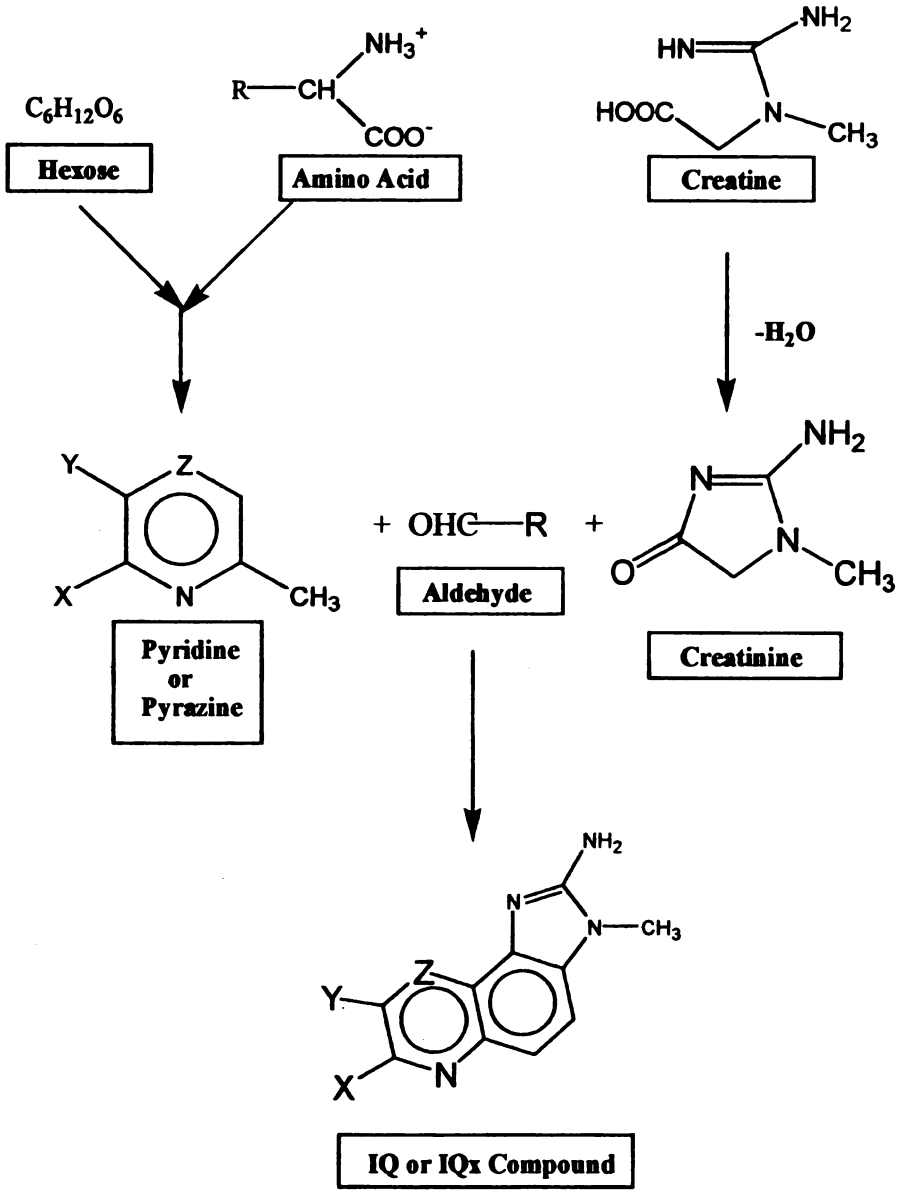


Figure 4. Theoretical reaction pathway for formation of IQ and IQx compounds. R, X, and Y may be H or CH_3: Z may be CH or N (Jägerstad et al., 1983b).

HAA formation in food and model systems (Botting et al., 1999).

Sugars and other carbohydrates

Sugars will impact HAA formation in food and model systems. Taylor et al. (1985) reported that glucose, when added to a beefstock supernatant at a concentration four times greater than that of creatine, reduced the overall mutagenicity of the heated system. Similarly, Skog and Jägerstad (1991) demonstrated that high amounts of sugar compared to the other two reactants (creatinine and phenylalanine) in model systems containing glucose, creatinine, and phenylalanine inhibited HAA formation. When sugars were present in equimolar or greater concentrations than creatin(in)e, HAA formation was notably reduced. Greatest mutagenicity was produced when the glucose level was one half that of creatinine or the amino acid. These results, observed only when amino acids were present in the reaction mixture, indicate a reaction between Maillard reaction products such as 5-hydroxymethyl-2-furfural (HMF) and creatinine. Thus, less creatinine would be available to form HAAs (Skog and Jägerstad, 1990).

The inhibitory effect of sugars, when added in excess, has also been studied in meat systems. Skog et al. (1992) demonstrated that glucose and lactose from milk powder, when added at concentrations up to 4 %, reduced mutagenicity by 34 to 76%. The greatest inhibitory effect was achieved by golden breadcrumbs when they were added in combination with glucose or lactose in meat systems.

Soy protein concentrate and defatted glandless cottonseed flour

Soy protein concentrates have been reported to reduce mutagenicity in fried beef patties by 24% (Wang et al., 1982). This reduction was attributed mainly to volumetric effects including the reduction of interactions among the beef components, and the reduction of the amount of beef that came into contact with the frying surface. Some consideration was given also to the possible inhibitory role of chlorogenic acid, a naturally occuring polyphenolic antioxidant in soy protein concentrates (Pratt and Birac, 1979; Rappaport et al., 1979; Smith and Circle, 1978). Another possible factor was the binding of soy protein concentrate with water, thus limiting the movement of the reactants to the surface of meat in contact with the frying system.

Defatted glandless cottonseed flour, when added at a level of 5% (w/w) to ground beef before frying, reduced the overall mutagenicity of the cooked meat (Rhee et al., 1987). As quercetin derivatives are the major flavonoids present in defatted glandless cottonseed, some consideration was given to their possible role as inhibitors of HAA formation. However, it was not clarified whether the reduction of mutagenicity was due to volumetric effects or to the intervention of specific cottonseed components.

Phenolic antioxidants

It has been shown that butylated hydroxyanisole (BHA) will reduce the mutagenicity of fried ground beef patties. Chen et al. (1992) confirmed the results of Wang et al. (1982) and further reported that BHA, propyl gallate and tertiary butylhydroquinone reduced the overall concentrations of IQ-like compounds in fried ground beef by approximately 80-90%. More recently, Faulkner (1994) reported that BHA (0.1% based on the fat content) reduced the mutagenicity in cooked beef patties

from 7000 revertants/100g raw meat to 2800 revertants/100g (raw meat basis) as determined by the Ames *S. typhimurium* assay.

Vitamin E has been extensively studied as an inhibitor of HAA formation in meat system. Chen et al. (1992) demonstrated that vitamin E (1% based on the fat content) reduced the concentration of IQ-like compounds by 50%. Balogh et al. (2000) reported that vitamin E (1% and 10% based on the fat content) reduced PhIP concentrations in fried beef patties by 69% and 72%, respectively. The direct addition of vitamin E (1% based on fat content) to the surface of the beef patties reduced MeIQx and MeIQ formation by 45% and 76%, respectively. These results clearly establish vitamin E as an effective inhibitor of HAA formation in ground beef patties.

Tea phenolics

Tea polyphenolic compounds such as epigallocatechin gallate (EGCG), epicatechin gallate, and epigallocatechin, have been established as potent antioxidants (Chen et al., 1990; Ho et al., 1992; Sichel et al., 1991; Sorata et al., 1984; Terao et al., 1984). The effects of black tea, green tea and the polyphenolics, theaflavine gallate (TFG) and EGCG, on the formation of HAAs were studied by Weisburger et al. (1994). They demonstrated reduction of HAA formation in model systems containing creatinine, glycine and glucose. Black tea had a substantial effect in lowering the formation of both MeIQx and PhIP (from 9350 to 7340 revertants/plate and from 6530 to 2070 revertants/plate, respectively). The effect of green tea in reducing the formation of mutagenic compounds was effective only for PhIP (from 6530 to 2180 revertants/plate). They also demonstrated that EGCG and TFG reduced mutagenic

activity from 74% to 83% for both MeIQx and PhIP. Oguri et al. (1998) investigated the inhibitory effect of green tea catechins and EGCG on formation of HAAs and their antimutagenicity activity. Green tea catechins and EGCG reduced MeIQx formation by 21% and 35%, respectively. The antimutagenic effects of green tea catechins and EGCG on MeIQx toward *S. typhimurium* TA98 were 29% and 46%, respectively.

Many studies have shown the relationship between antioxidant activity and antimutagenicity of green tea, pouching tea, oolong tea and black tea (Yen and Chen, 1995). The antimutagenic effect of tea extracts on IQ toward *S. typhimurium* TA98 and TA100 was correlated with their reducing power and scavenging effect on the hydroxyl radical. The antioxidant effect of tea extracts was well correlated to their antimutagenicity in some cases, but varied with the mutagenic and antioxidative properties. Dashwood et al. (1999) showed that both green tea (2% w/v) and black tea (2% w/v) reduced mutagenicity against the IQ compound. *N*-hydroxy-IQ, a direct-acting mutagen in the Ames assay, was inhibited by individual components of tea such as epigallocatechin-3-gallate (EGCG) and epigallocatechin (EGC). Further, they also demonstrated the modulation effect on HAA metabolism. NADPH-cytochrome P450 reductase and *N,O*-acetyltransferase, enzymes for metabolic activation of HAAs, were inhibited by tea. Studies *in vivo* established that tea also induced cytochrome P450 and Phase II enzymes with the rapid metabolism and excretion of HAAs.

History and Therapeutic Effects of Garlic

Garlic has been cultivated since antiquity and has been used as foodstuffs and medicines. Botanists call it *Allium sativum* L., and the origin of the genus name

remains unknown. It is possibly derived from Latin word *olere*, "to smell" because of its strong odor or it is derived from the Greek word *hallestai*, "to leap out" (Milner, 1996). Garlic has approximately 600 known species distributed over Europe, North America, North Africa, and Asia, and the cultivated form of garlic, *Allium sativum* L., presumably originated from central Asia (Lawson, 1996).

Large amounts of garlic are produced in Egypt, India, China, and South Korea. Recently, China has become a major user and producer of garlic. Argentina is the notable main producer in South America. The "Garlic Capital" of the U.S. is Gilroy, CA because it produces around 80 to 90% of the garlic consumed in the U.S. Garlic has captured a secure popular position with consumers due to its various alleged therapeutic effects and common use. It was formerly popular, and still is, to some extent as a carminative for dyspeptic problems and diarrhea, an antimicrobial for bacteria, a fungal, and viral infections, as well as a vermifuge for intestinal parasites (Reuter and Sendl, 1994). In recent years, garlic has become highly valued because of its excellent effectiveness toward arteriosclerosis, its ability to lower serum cholesterol and triglyceride levels, and its hypotensive, anticarcinogenic, and antidiabetic effects (Kritchevsky, 1991). Garlic may also inhibit thrombocyte aggregation and activate fibrinolysis (Kendler, 1987; Orth-Wagner, 1986; Weiss, 1986). The U.S. Department of Agriculture reported that U.S. garlic consumption in 1989 was 1.0 pounds per capita, and it has soared to 3.1 pounds in 1999 (Lucier and Lin, 2000).

The composition of garlic

The composition and chemistry of garlic has been extensively reviewed because of its popularity and various health benefits. Garlic has been sold in various forms such as bulbs, picked cloves, crushed or chopped cloves, spice powders, and garlic salts. Also, garlic supplements are sold as powder tablets or capsules with various coatings. Due to chemical and enzymatic changes that take place during processing or preparation steps, different forms of garlic contain different compounds (Lawson, 1996). The general composition of garlic is shown in Table 4. The water content of garlic is about 65%, which is relatively lower than most fruits and vegetables (80-90%). The bulk of the dry weight is composed of carbohydrates, sulfur compounds, protein, and free amino acids (Lawson, 1996).

Sulfur compounds in garlic

Many garlic studies have focused on the sulfur compounds because of their high concentrations relative to those in fruits and other vegetables (Figure 5). Various reports have cited the health promoting effects of sulfur compounds including antibacterial (Deshpande et al., 1993; Hughes and Lawson, 1991; Shashikanth et al., 1986), antifungal (Ghannonoum, 1988; Hughes and Lawson, 1991; Yoshida et al., 1987), antiarteriosclerotic (Mohammad and Woodward, 1986), antithrombotic (Lawson et al., 1992), and blood lipid-lowering activities (Kamanna and Chandrasekhara, 1984; Plengvidhya et al., 1988; Sitprija et al., 1987).

A German scientist, Wertheim (1844), discovered that steam distillation of crushed garlic produced strong smelling oil which consisted exclusively of organosulfur

Table 4. The general composition of garlic.

Component	Amount (%fresh weight)
Water	62-68
Carbohydrates	26-30
Protein	1.5-2.1
Amino acids: common	1-1.5
Amino acids: cysteine sulfoxides	0.6-1.9
y-Glutamylcysteine	0.5-1.6
Lipids	0.1-0.2
Fiber	1.5
Total sulfur compounds	1.1-3.5
Sulfur	0.23-0.37
Nitrogen	0.6-1.3
Minerals	0.7
Vitamins	0.015
Saponins	0.04-0.11
Total oil-soluble compounds	0.15 (whole)-0.7 (cut)
Total water-soluble compounds	97

Table compiled using information from Lawson et al. (1991), Lawson (1993), Pentz et al. (1990), and Ueda et al. (1991).

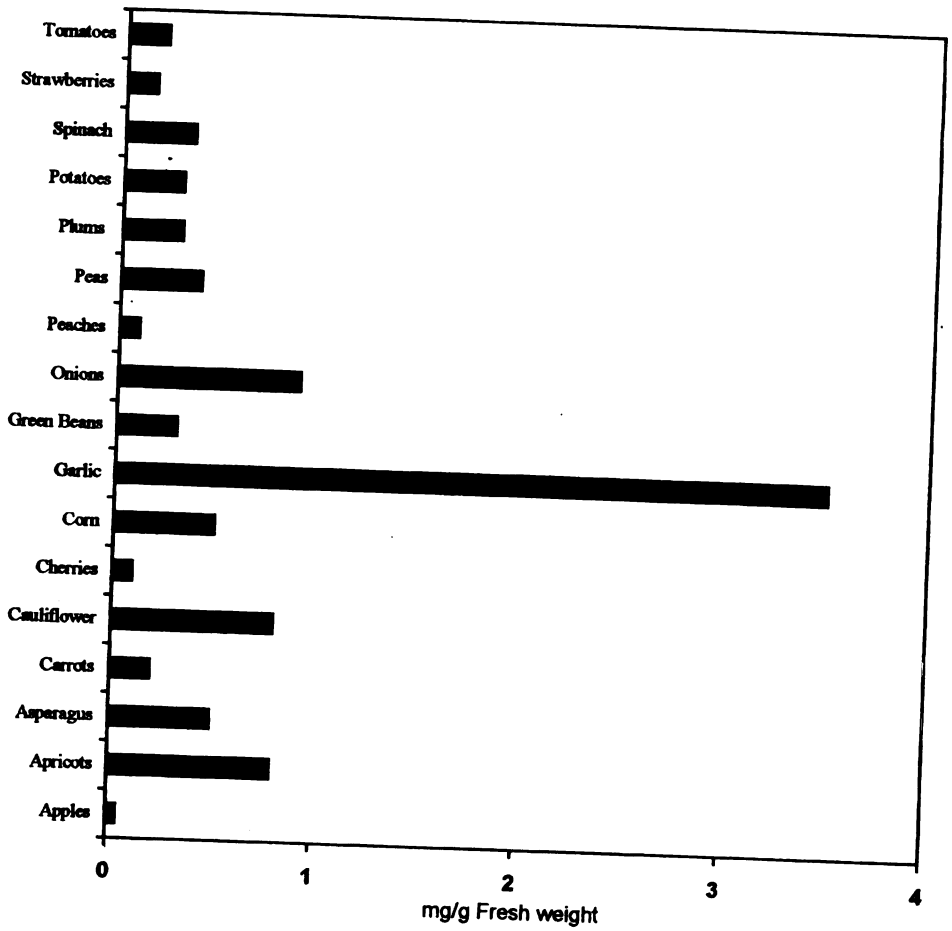


Figure 5. Sulfur content of common fruits and vegetables (Nielson et al., 1991).

compounds. He also determined these compounds had a basic formula of $C_6H_{10}S$ and named the hydrocarbon group "*allyl*" after *Allium sativum*. However, almost 50 years later, Semmler (1892) fractionally distilled the oil and identified specific compounds. He correctly determined the formula of allyl to be C_3H_5 instead of C_6H_{10} and found the oil contained 60% diallyl disulfide (DAD), 20% diallyl trisulfide, 10% diallyl tetrasulfide, and 6% allyl propyl disulfide. The allyl propyl disulfide content was later shown to be an error since *S*-propyl compounds are not produced by garlic (Block et al., 1992, 1993; Lawson et al., 1991).

Almost 50 years after Semmler's work, Cavallito and colleagues (Cavallito and Bailey, 1944; Cavallito et al., 1944, 1945) reported that the antibacterial activity of crushed garlic clove was due to the presence of an oxygenated sulfur compound which possessed the odor of freshly cut garlic. This compound was named allicin. Cavallito et al. (1945) concluded that it must be formed on crushing garlic, and it was the precursors of the diallyl sulfides present in the oil of steam-distilled garlic. Stoll and Seebeck (1947) reported that alliin, the parent compound of allicin, had no antibiotic activity unless converted to allicin by an enzyme, alliinase. The thiosulfinates present in crushed garlic are transformed at room temperature to diallyl trisulfide, diallyl disulfide, and allyl methyl trisulfide (Iberl et al., 1990). Work in Finland on onions (Virtanen, 1965, 1969; Virtanen et al., 1992) and ^{35}S-labeled garlic studies (Sugii et al., 1964; Suzuki et al., 1961, 1962), indicated that garlic contains nine different γ-glutamylpeptides, six of which contain the sulfur containing amino acid, cysteine. The most abundant γ-glutamylcysteine in garlic, γ-glutamyl-S-trans-propenylcysteine, was not discovered until almost 30 years later (Lawson and Hughes, 1990; Lawson et al.,

1991). The sulfur content of garlic is approximately 1.0% of its dry weight (0.35% of its fresh weight) (Pentz et al., 1990; Ueda et al., 1991). Alliin, allicin, and the two main γ-glutamylcysteines, constitute the majority (about 72%) of the sulfur compounds in whole or crushed garlic. Sixteen nonprotein organosulfur compounds have been found in whole cloves and 23 in crushed cloves (Table 5). Three minor compounds (methionine, γ-glutamylmethionine, and thiamine) out of the 16 nonprotein organosulfur compounds present in whole cloves do not contain the amino acid cysteine. Figure 6 shows the formation of allicin by the action of alliinase and the transformation of the principal thiosulfinates of crushed garlic.

The minor organosulfur compounds consist mainly of methyl and 1-propenyl homologs of alliin and allicin, and γ-glutamyl-S-methylcysteine (which comprises about 13% of the total sulfur). Trace amounts (less than 0.1 mg/g) of a few other related compounds (8 in whole cloves, 11 in crushed cloves) are also found. Protein-soluble and insoluble-sulfur, and inorganic sulfate, comprise about 9% and 5% of the total sulfur (Lawson, 1993). The sulfur compounds of garlic constitute about 86% of the total sulfur of garlic cloves and about 98±2% of the total nonprotein organosulfur compounds.

Role of Sulfur Compounds in the Biotransformation of Xenobiotics and the Inhibition of Heterocyclic Aromatic Amine Formation

Sulfur compounds have been reported to play a key role in the biotransformation of xenobiotics by actively participating in their detoxification and also by inhibiting the

Table 5. Sulfur compounds in whole and crushed garlic cloves (Lawson, 1996).

Compound	Whole Garlic	Crushed Garlic
	(mg/g fresh weight)	
S-(+)-Alk(en)yl-L-cysteine Sulfoxides		
S-Allylcysteine sulfoxide (alliin)	5-14	ndb
S-Methylcysteine sulfoxide (methiin)	0.5-2.0	nd
S-trans-1-Propenylcysteine sulfoxide (isoalliin)	0.2-1.2	nd
S-Propylcysteine sulfoxide	nd	nd
Cycloalliin	0.5-1.5	0.5-1.5
γ-L-Glutamyl-S-alk(en)yl-L-cysteines		
γ-Glutamyl-S-trans-1-propenylcysteine	3-9	3-9
γ-Glutamyl-S-cis-1-propenylcysteine	0.06-0.15	0.06-0.15
γ-Glutamyl-S-allylcysteine	2-6	2-6
γ-Glutamyl-S-methylcysteine	0.1-0.4	0.1-0.4
γ-Glutamyl-S-propylcysteine	nd	nd
Thiosulfinates		
	nd	2-6
Allyl 2-propenethiosulfinate (allicin)	nd	0.3-1.5
Ally methyl thiosulfinates	nd	0.05-1.0
Ally trans-1-propenylthiosulfinates	nd	0.02-0.2
Methyl trans-1-propenylthiosulfinate	nd	0.05-0.1
Methyl methanethiosulfinate		
Others	nd	nd
	nd	nd
Cysteine	nd	nd
Cystine	nd	nd
Glutathione, reduced	nd	nd
Glutathione, oxidized	nd	nd
γ-Glutamyl-S-allycysteine sulfoxide	nd	nd
γ-Glutamyl-S-trans-1-propenylcysteine sulfoxide	0.02-0.12	0.02-0.12
γ-Glutamyl-S-methylcysteine sulfoxide	nd	nd
γ-Glutamy-methionine	tr	tr
γ-Glutamylsysteine, reduced	0.09	0.09
γ-Glutamycysteine, oxidized	0.01-0.03	0.01-0.03
S-2-carboxypropylglutathione	tr	tr
γ-Glutamyl-S-allymercaptocysteine	nd	nd-0.006
S-Methycysteine	nd-0.026	nd-0.026
S-1-Propenyl cysteine	nd	0.002
S-Allylcysteine	0.02	0.02
S-Allylmercaptocysteine	0.002	nd-0.001
Methionine	nd	nd-0.001
Thiamine	0.03 (yield)	<0.03
Allithiamine	nd, 0.01	nd, 0.01
Scordinins	0.3	0.3
Sulfolipids	0.6	0.6
Protein, soluble	0.5	0.5

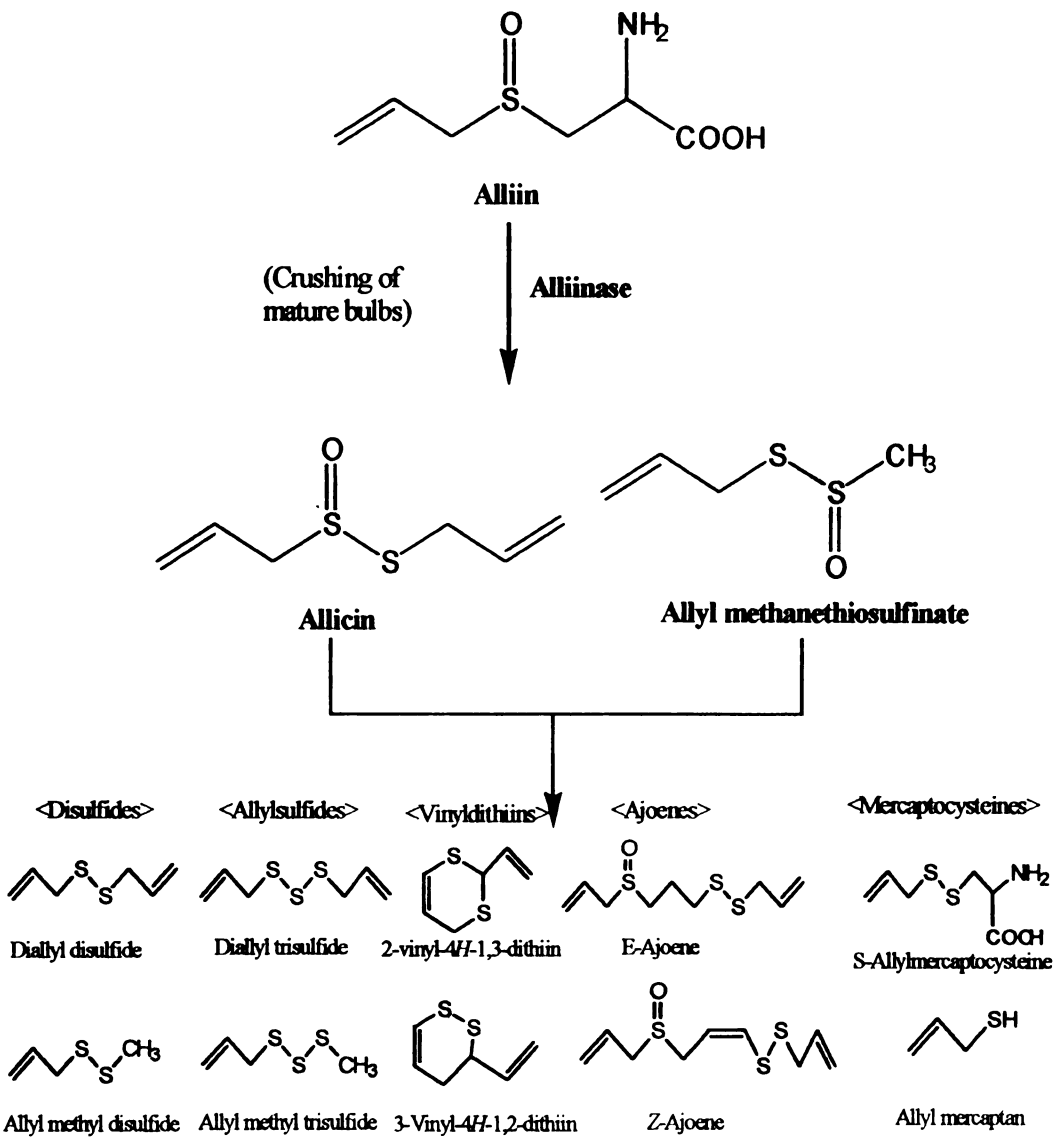


Figure 6. Formation of allicin by the action of alliinase and transformation of the principal thiosulfinates of crushed garlic (Block et al., 2001).

action of mutagens, carcinogens, and other toxic compounds (Friedman and Molnar-Perl, 1990). Many studies have shown the effect of sulfur compounds on the biotransformation of xenobiotics by detoxification and modulation of mutagenesis, carcinogenesis, and other toxic compound metabolism (Sheen et al., 1999). DAD inhibited benzo[*a*]pyrene-induced forestomach tumors, pulmonary adenoma and skin carcinogenesis (Singh and Shukla, 1998; Sparnins et al., 1988). Surh et al. (1995) demonstrated that DAD reduced vinyl carbamate and N-nitrosodimethylamine (NDMA)-induced mutagenesis which correlated with inhibition of P450 2E1-mediated *p*-nitrophenol hydroxylation and NDMA *N*-demethylation. Friedman et al. (1982) reported that cysteine and other related thiols inactivated the mutagenecity of aflatoxin in *in vitro* experiments. De Flora (1989) showed that the *N*-acetyl-cysteine dramatically decreased urethane-induced tumor formation in mice. Troll (1986) reported that a sulfur-rich protein called the Bowman-Birk inhibitor suppressed N-nitrosamine-induced carcinogenicity in the digestive tract of rats. They suggested that the possible effectiveness of garlic sulfur compounds in preventing the formation of toxic compounds is by trapping intermediates, and by preventing activation of biologically active forms.

There have been several reports of HAA inhibition in meats by onion and garlic extracts. Kato et al. (1998) reported reduction of mutagen formation (74%) in hamburger by the addition of onion juice. Murkovic et al. (1998) found that garlic reduced IQ, MeIQ, MeIQx, Di MeIQx, and PhIP formation in fried ground beef by 32%, 71%, 40%, 78%, and 54%, respectively. They suggested that the effect of garlic may be due to sulfur compounds in the essential oil of garlic. Salmon et al. (1997)

demonstrated that marinating chicken breasts for 20 min before grilling reduced PhIP formation by 92-99%. While the marinade contained olive oil, brown sugar, cider vinegar, lemon juice, garlic, salt, and mustard, the inhibitory effect was not assigned to any particular ingredient. Tsai et al. (1996) demonstrated that addition of sulfur compounds (DAD and dipropyl disulfide (DPD)) to a pork meat juice model system before heating reduced the formation of Maillard reaction products which correlated with a decrease in mutagenicity. They reported that sulfur compounds showed various dose-dependent responses in reducing the mutagenicity of extracts of boiled pork juice by inhibiting IQ mutagen formation.

Recently, Trompeta and O'Brien (1998) reported that various sulfur compounds including glutathione, L-cysteine, L-cystine, and deoxyalliin inhibited the formation of mutagenic compounds in a model system containing glucose, glycine, and creatinine which was heated in a diethylene glycol reflux system. They suggested several possible mechanisms of inhibition of HAA formation by sulfur compounds. These compounds could act as reducing agents, scavengers of free radicals, strong nucleophiles which can trap electrophilic compounds and intermediates, precursors for intracellular reduced glutathion, and inducers of cellular detoxification. These investigators further proposed that addition of sulfur compounds could possibly lead to decreasing amounts of HAA formation through the competitive reaction between sulfur compounds and amino acids for glucose. This proposition is supported by the results of Arvidsson et al. (1999), which showed the rapid depletion of glucose in the early stages of the reaction.

Sulfur compounds have been shown to react with glucose to produce several of volatile compounds (Yu et al., 1994). Thiazoles, especially 2-acetylthiazole, were

predominant volatile interaction products of alliin and glucose, whereas pyrazines, especially 2,5-dimethyl-, methyl-, and trimethylpyrazine, were found to be the predominant volatile interaction products of deoxyalliin and glucose.

CHAPTER TWO

Inhibition of heterocyclic aromatic amine formation in fried ground beef patties by garlic and its sulfur compounds

ABSTRACT

The effects of garlic and selected organosulfur compounds (diallyl disulfide, dipropyl disulfide, diallyl sulfide, allyl methyl sulfide, allyl mercaptan, cysteine, and cystine) on the formation of heterocyclic aromatic amines (HAAs) in fried ground beef patties were evaluated. Minced garlic cloves (approximately 4.8-16.7% w/w) or organosulfur compounds (0.67 mmol) were added directly to ground beef. Patties (100g) were fried at 225°C (surface temperature) for 10 min per side. Two patties were fried for each replication, and five replicates were analyzed for each treatment. For each replicate, four sub-samples were analyzed (two unspiked for concentration and two spiked for recovery of HAA standards). The volatile sulfur compounds significantly ($p < 0.05$) reduced the concentrations of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*] pyridine (PhIP) (46-81% reduction), while average reductions of 35, 22, and 71%, were achived by cystine, cysteine, and whole garlic, respectively. Concentrations of 2-amino-3,8-dimethylimidazo-[4,5-*f*]quinoxaline (MeIQx) were reduced 34 to 67% by the volatile sulfur compounds, and 25, 19, and 63% ($p<0.05$) by cystine, cysteine, and whole garlic, respectively. These studies confirm that garlic and some organosulfur compounds have the potential to reduce HAA formation in cooked beef patties.

INTRODUCTION

The human diet is a complex mixture of organic and inorganic substances that not only provide nutritional benefits, but also play a role in the causation and modulation of human health risk (Thomson, 1999). Many mutagens and carcinogens have been isolated and identified in foods. Recently, several foods and constituents of foods have been investigated for their inhibitory or promotional effects on carcinogenesis (Knize et al., 1999; Weisburger, 1991).

Heterocyclic aromatic amines (HAAs) are formed in muscle foods when cooked at temperatures generally in excess of 150°C. Several have been identified in cooked fish and meat and are believed to result from the interaction of creatine/creatinine, sugars and free amino acids. These compounds, also called aminoimidazoazaarenes, can be broken down into four categories: quinolines, quinoxalines, pyridines, and furopyridines. The most commonly found HAAs in foods are IQ (2-amino-3-methylimidazo[4,5-*f*]-quinoline); MeIQ (2-amino-3,4dimethylimidazo[4,5-*f*]-quinoline); MeIQx (2-amino-3,8 dimethylimidazo [4,5-*f*]-quinoxaline); 4,8 DiMeIQx (2-amino-3,4,8 trimethylimidazo[4,5-*f*]-quinoxaline); and PhIP (2-amino-1-methyl-6-phenylimidazo [4,5-*b*]-pyridine) (Knize et al., 1999; Skog, 1993; Wakabayashi and Sugimura, 1998).

Cooking time and temperature, and method of cooking, all influence the formation of HAAs in cooked meat products (Knize et al., 1994, 1999). Generally, those methods where meat is in direct contact with the heating surface or flame, such as in pan-frying and grilling, produce the greatest quantities of HAAs. The formation of HAAs in cooked meat can be reduced by the addition of selected compounds or

ingredients such as vitamin E (Balogh et al., 2000), cherry tissue (Britt et al., 1998), tea polyphenolic compounds (Yen and Chen, 1995), soy protein concentrate (Wang et al., 1982) and defatted glandless cottonseed flour (Rhee et al., 1987).

Garlic has been cultivated since antiquity and has been used as a foodstuff and for medical purposes. A variety of garlic-based health products are now readily available on the market. In recent years, garlic has become highly valued because of its impact on arteriosclerosis (Kritchevsky, 1991), its ability to lower serum cholesterol (Reuter and Sendl, 1994) and triglyceride levels (Weiss, 1986), and for its hypotensive (Kendler, 1987), anticarcinogenic (Wattenberg et al., 1989) and antidiabetic properties (Augusti and Sheela, 1996). Garlic also inhibits thrombocyte aggregation (Nishimura et al., 1988) and activates fibrinolysis (Weiss, 1986). Many food preparation methods in the home include the cooking of meats with various vegetables and spices that contains organosulfur compounds. However, there is not much information on the effect of these compounds on HAA formation during the cooking of meats. The objective of this study was to evaluate the effectiveness of garlic and individual organosulfur compounds present in garlic as inhibitors of HAA formation in cooked ground beef patties.

MATERIALS AND METHODS

Safety

Heterocyclic aromatic amines are mutagenic/carcinogenic and should be handled with appropriate safety precautions including the use of goggles, latex gloves and efficient fume hoods.

Materials

Diallyl disulfide (DAD), dipropyl disulfide (DPD), diallyl sulfide (DAS), allyl methyl sulfide (AMS), allyl mercaptan (AM), cysteine, and cystine were purchased from Fluka Chemical Co. (Buchs, Switzerland). The HAA standards (MeIQx, 4,8-DiMeIQx, and PhIP) were obtained from Toronto Research Chemicals (Toronto, Canada). The HAA standard (FEMA-Flavor and Extracts Manufacturer's Association) and the internal standard, caffeine, were gifts from Dr. Mark Knize, Lawrence Livermore National Laboratory, Livermore, CA. The FEMA standard contained IQ, MeIQ, MeIQx, DiMeIQs, and PhIP, each at 5 ng/μL. Propyl-sulfonic acid (PRS) Bond-Elut columns (500 mg) and C18 cartridges (100 mg) were purchased from Varian Inc. (Harbor City, CA). Extrelut-20 columns and Extrelut diatomaceous earth were obtained from E.M. Separations Technology (Gibbstown, NJ). All other chemicals were of analytical grade and were purchased from Fisher Scientific (Fair Lawn, NJ).

Freshly ground beef (approximately 15% fat) was obtained from a local supermarket and used within one hour of purchase, or stored at -20°C until required for

frying. Fat content was determined by the method of Folch et al. (1957). Fresh garlic was also purchased from a local supermarket.

Preparation of ground beef patties

Patties were prepared from ground beef as follows: control patties (ground beef patties containing 1 mL of methanol) and patties containing various quantities of minced garlic cloves and organosulfur compounds added as separate treatments two hours prior to frying. Minced garlic cloves (4.5 - 18.2%, w/w) or organosulfur compounds (0.17 - 1.01 mmol DAD, DPD, DAS, AMS, AM, cysteine, and cystine) were mixed/dissolved in 1 mL methanol and added directly to the ground beef, and then mixed in a Keebler mixer (Keebler Inc., Chicage, IL) for 2 min. Each patty (100g) was formed by placing the ground beef in a petri dish (9 cm dia. × 1.5 cm thickness) to ensure patty uniformity. The study was repeated three to five times using one batch of ground beef with a fat content of 15.4±2.0%.

Cooking of patties

Patties were fried in a teflon-coated electric frying pan at 225°C (surface temperature) for 10 min per side. The temperature of the frying surface was measured using a surface temperature thermometer (Pacific Transducer Corp., Los Angeles, CA). Two patties were fried for each replication, and five replicates were analyzed for each treatment. For each replicate, four subsamples were analyzed (two unspiked for concentration and two spiked for recovery). The cooked meat patties were mixed in a blender to produce a uniform sample, and frozen at -4°C until extraction.

Quantification of HAAs in ground beef patties

Concentrations of HAAs in the beef patties were determined by the standard addition method of Gross and Grüter (1992). Meat samples were extracted by homogenizing 30 g cooked meat with 90 g 1N NaOH. The homogenate was divided into four equal aliquots. To determine extraction recoveries, two of the aliquots were spiked with 250 ng each of IQ, MeIQ, MeIQx, DiMeIQx and PhIP dissolved in 50 µl methanol. Samples were mixed with Extrelut diatomaceous earth to fill an Extrelut 20 column. All four extractions were made with 40 mL dichloromethane containing 5% toluene using attached Bond Elut PRS extraction columns. The PRS cartridges were washed sequentially with 6 mL 0.1N HCL, 15 mL 40% methanol in 0.1N HCL, and 2 mL water. The HAAs were then transferred to Bond Elut C18 cartridges (100mg) with 20 mL ammonium acetate buffer solution (0.5 M, pH 8.0). The cartridges were eluted with 0.8 mL MeOH:NH_4OH (9:1, v/v). The elute was evaporated to dryness, and the residue dissolved in 50 µL methanol containing 5 ng/µL caffeine as an internal standard.

Separation of the HAAs was carried out on a TSK-gel ODS80-TM column (25 cm × 4.6 mm id; Tosoh Haas, Montgomeryville, PA). A precolumn (Supelguard LC-8-DB, Supelco, Bellefonte, PA) was attached between the injector port and column to filter out unwanted compounds, and the cartridge was replaced approximately every 60 injections. The flow rate of the mobile phase was 1 ml/min. The initial ratio of acetonitrile:buffer (triethylamine phosphate, 0.01 M, pH 3.2) was 8:92, which increased to 17:83 during the first 10 min. The acetonitrile concentration continued to increase until the ratio was 25:75 (10 min), then 55:45 (10 min). Over the next five min, the

acetonitrile:buffer ratio was increased to 80:20 to facilitate elution of other compounds. After 35 min, the eluting solvent was returned to its initial ratio (8:92) for 10 min to allow the column to re-equilibrate before the next injection. Samples were analyzed on a Millennium 2000 HPLC system (Millipore, Milford, MA) with a photodiode array detector (Model 991) and a scanning fluorescence detector (Model 474).

The identities of the peaks were established by comparing retention times of the peaks with those of the corresponding spiked samples analyzed under the same conditions. Furthermore, UV spectral characteristics of the HPLC peaks in each sample were compared with library spectra acquired from standard HAA solutions. For each experiment, before HPLC separation of the sample extracts, four aliquots (10, 15, 20 and 25 μL) of two standard mixtures of HAAs (containing 0.5 ng/μL of each compound), the caffeine internal standard (5 ng/μL), and HAA standard FEMA (5 ng/μL) were injected. Linear regression (ng compound *vs* peak area) was performed for individual HAAs in each mixture. A correlation coefficient of 0.99 or greater was considered acceptable for FEMA internal standards, and 0.97 or greater for the laboratory mixtures of HAAs. Each peak area corresponding to an HAA was corrected with the internal standard regression line and expressed as ng/g meat. The standard addition method of Gross and Grüter (1992) was then used for determining extraction efficiency and for quantification of HAAs. Each data point consisted of four subsamples; two spiked and two unspiked. The average area of the spiked samples minus the average of the unspiked samples allowed comparison with the regression line for the standard mixture. Each data point was then corrected for its individual extraction efficiency, or percent yield. Concentrations of each HAA formed were

determined using the average of the two unspiked subsamples. The linear regression slope for FEMA was used used to determine the exact amount of each HAA present in each sample.

Statistical analyses

The results were analyzed by Sigma Stat 2.0 (Jandel Corp., San Rafael, CA). One-way analysis of variance (ANOVA) was performed for each HAA. Appropriate comparisons were made using Student-Newman-Keuls test for one-way ANOVA analysis (Neter et al., 1993).

RESULTS AND DISCUSSION

In an initial study, ground beef patties were fried at 180°C for 10 min per side. However, these cooking conditions produced little or no HAA formation in the control patties or in those containing the whole garlic or sulfur compounds. Consequently, patties were fried at 225°C for 10 min per side to generate sufficient quantities of HAAs to evaluate the inhibitory effects of garlic and selected organosulfur compounds on HAA formation. The dominant HAA in fried ground beef patties was PhIP, followed by MeIQx, and DiMeIQx. IQ and MeIQ are found infrequently in cooked beef, and at very small concentrations (Skog et al., 1995). The quantification of IQ and MeIQ compounds in cooked beef is problematic because of difficulties with co-elution and peak interference (Skog et al., 1995). Average recoveries of HAAs added to the cooked ground beef patties were 74±16, 74±19, and 66±16% for MeIQx, Di MeIQx, and PhIP,

respectively. These recoveries are comparable to those reported by Knize et al. (1997) and Britt et al. (1998). Salmon et al. (1997) reported recoveries ranging from 35 to 98% for IQ, MeIQx and DiMeIQx and from 9 to 63% for PhIP, while Britt et al. (1998) reported average recoveries of 84%, 73%, and 65% for MeIQx, Di MeIQx, and PhIP, respectively.

Minced garlic, when added at levels of 4.8, 9.1, 13.0, and 16.7%, inhibited HAA formation (i.e., the sum of the concentrations of MeIQx, DiMeIQx and PhIP) by 28, 59, 63, and 68%, respectively (Table 1). Analysis of variance revealed that the addition of minced garlic at levels greater than 9.1% significantly ($p < 0.05$) reduced the formation of HAAs in fried ground beef patties. Statistical analysis also revealed no significant difference among the percentages of inhibition by these levels of minced garlic (Table 1).

Several literature reports allude to HAA reduction by the addition of garlic and onion to meat products before cooking. Murkovic et al. (1998) reported that rosemary, thyme, sage, garlic, and brine reduced PhIP concentrations in fried ground beef by 39, 100, 64, 78, and 100%, respectively. Salmon et al. (1997) demonstrated that marinating chicken breast 20 min before grilling reduced PhIP formation by 92-99%. While the marinade contained olive oil, brown sugar, cider vinegar, lemon juice, garlic, salt, and mustard, the inhibitory effect was not assigned to any particular ingredient. Kato et al. (1998) reported that onion juice reduced mutagenicity of cooked ground beef and concluded that the sugars present in onion juice were responsible for the inhibitory action. Fresh onion contains 7.6% (w/w) sugar. On a dry matter basis, onion contains 33-44g (w/w) of soluble sugars (fructose, glucose, and sucrose) per 100g dry matter.

Table 1. Effect of minced garlic cloves on the formation of heterocyclic aromatic amines in ground beef patties[1]

	Heterocyclic aromatic amines (ng/g)[2]				
Treatment	**MeIQx**	**DiMeIQx**	**PhIP**	**Total HAAs**	**Inhibition (%) of total HAA formation**
Control	5.3±0.9[a]	2.9±0.5[a]	13.5±2.1[a]	21.7	
5g Garlic	4.6±0.8[a]	2.2±0.4[a]	8.9±1.1[a]	15.7	28
10g Garlic	2.7±0.6[b]	1.3±0.3[b]	5.0±1.1[b]	8.9	59
15g Garlic	2.4±0.6[b]	1.2±0.3[b]	4.4±0.9[b]	9.0	63
20g Garlic	2.0±0.8[b]	1.1±0.2[b]	3.9±1.0[b]	6.9	68

[1] Values are based on measured cooking losses for individual patties.
[2] Means with the same superscript are not significantly different ($p>0.05$).
Data are the means of three replicates.

The inhibitory effect of sugars on HAA formation has been reported by other investigators. Skog et al. (1992) demonstrated that glucose and pure lactose or lactose from milk powder, when added to beef patties at concentrations up to 4 %, reduced mutagenicity by 34 to 76%. On the other hand, garlic has a sugar content of only 0.1% (w/w) (Lawson, 1993), and thus we conclude that the inhibitory effect of garlic is not likely to be due to the presence of sugars.

The addition of DAD to ground beef patties at concentrations of 0.17, 0.34, 0.67, 0.84, and 1.01 mmol inhibited total HAA formation by 45, 68, 78, 83, and 84%, respectively (Table 2). DAD, when added at 0.67 mmol and higher, significantly ($p <$ 0.05) reduced MeIQx, DiMeIQx and PhIP in fried ground beef patties. There was, however, no significant difference in the percent inhibition achieved by these concentrations of DAD (Table 2). At the 0.67 mmol concentration, DAD reduced the total HAA concentration in the cooked patties from 21.2 to 4.7 ng/g meat (Table 2). These results agree with those of Tsai et al. (1996) who reported that DAD and DPD reduced the overall mutagenicity of boiled pork juice by 80 to 98%, respectively. Trompeta and O'Brien (1998) also demonstrated that selective organosulfur compounds such as glutathione, L-cysteine, L-cystine, and deoxyalliin inhibited HAA formation in a model system containing glucose, glycine, and creatinine. Based on the results of this initial study and on literature observations, several sulfur compounds (DAS, AMS, AM, DPD, cystine, and cysteine) were chosen for further investigation as inhibitors of HAA formation in cooked ground beef patties.

The addition of DAD and DPD (0.67 mmol) to ground beef patties inhibited PhIP formation by 81% and 69%, respectively (Table 3). DAD and DPD also inhibited

DiMeIQx formation in fried ground beef patties by 79% and 62%, respectively. The inhibitory effects of DAD and DPD were greater than those afforded by cysteine and cystine and the other volatile sulfur compounds that were evaluated. Similar observations were reported by Tsai et al. (1996). Why greater inhibition was achieved with DAD and DPD than cysteine and cystine was not explained by these investigators. The volatile disulfide afforded more effective inhibition than the other compounds which may be explained by the possible scission of the disulfide bond during heating to provide sulfhydryl groups. These groups have been implicated in the Maillard reaction (Friedman and Molnar-Perl, 1990), a reaction which has been postulated to be the involved in the formation of HAAs (Jägerstad et al., 1983a).

How sulfur compounds such as DAD and DPD inhibit HAA formation has not been investigated in any detail. However, a possible mechanism for the inhibition is through a competitive reaction between sulfur compounds and amino acids for glucose, a key component in the reactions leading to HAA formation. Mottram and Whitfield (1995) reported that sulfur compounds may directly participate in Maillard reactions leading to meat flavor production. Alliin and deoxyalliin have also been shown to react with glucose in Maillard reactions (Yu et al., 1994). Tsai et al. (1996) showed that the addition of sulfur compounds to a pork meat juice system reduced the formation of Maillard reaction products and this reduction was correlated with a decrease in mutagenicity. Further studies are necessary to evaluate the mechanism of the inhibition of HAA formation by organosulfur compounds.

Table 2. Effect of variable diallyl disulfide concentrations on the formation of heterocyclic aromatic amines in ground beef patties[1]

	Heterocyclic aromatic amines (ng/g)[2]				
Treatment	MeIQx	DiMeIQx	PhIP	Total HAAs	Inhibition (%) of total HAA formation
Control	5.0±0.6[a]	2.6±0.2[a]	13.6±1.4[a]	21.2	
0.17 mmol DAD	3.6±0.3[a]	1.4±0.2[a]	6.7±0.9[b]	11.8	45
0.34 mmol DAD	2.5±0.3[b]	1.0±0.1[a]	3.3±0.4[c]	6.8	68
0.67 mmol DAD	1.7±0.2[b]	0.5±0.1[b]	2.5±0.3[c]	4.7	78
0.84 mmol DAD	1.6±0.2[b]	0.5±0.1[b]	1.4±0.2[c]	3.5	83
1.01 mmol DAD	1.5±0.1[b]	0.5±0.1[b]	1.4±0.2[c]	3.4	84

[1] Values are based on measured cooking losses for individual patties.
[2] Means with the same superscript are not significantly different ($p>0.05$). Data are the means of three replicates.

Table 3. Effect of various sulfur compounds on the formation of heterocyclic aromatic amines in ground beef patties[1,2]

	Heterocyclic aromatic amines (ng/g)[3]		
Treatment	MeIQx	DiMeIQx	PhIP
Control	5.23±0.9[a]	2.9±0.5[a]	13.5±2.1[a]
DAD	1.7±0.2[f]	0.6±0.2[e]	2.6±1.1[d]
Inhibition (%)	67	79	81
DAS	2.8±0.3[e]	1.3±0.5[d]	5.5±1.2[c,d]
Inhibition (%)	46	55	59
AMS	3.3±0.3[d]	1.5±0.5[d]	6.4±1.3[b,c]
Inhibition (%)	38	49	52
AM	3.5±0.5[d]	1.7±0.5[c,d]	7.3±1.5[b,c]
Inhibition (%)	34	43	46
DPD	2.6±0.6[e]	1.1±0.4[d]	4.2±0.9[c,d]
Inhibition (%)	51	62	69
Cystine	4.0±0.6[c]	2.0±0.5[b,c]	8.8±1.3[b]
Inhibition (%)	25	32	35
Cysteine	4.23±0.53[b]	2.3±0.5[b]	10.5±1.4[b]
Inhibition (%)	19	22	22

[1] Values are based on measured cooking losses for individual patties.
[2] Sulfur compounds were added at 0.67 mmol level to ground beef patties.
[3] Means with the same superscript are not significantly different ($p>0.05$). Data are the means of five replicates.

CHAPTER THREE

Reduction of heterocyclic aromatic amine formation and mutagenicity in fried ground beef patties by organosulfur compounds

ABSTRACT

The effects of several organosulfur compounds on heterocyclic aromatic amine (HAA) formation in fried ground beef patties and overall mutagenicity of the patties were evaluated. Organosulfur compounds were added directly to ground beef and thoroughly blended. Patties weighing 100g were fried in a teflon-coated electric frying pan at 225°C (surface temperature) for 10 min per side. Two patties were fried for each replication, and five replicates were analyzed for each treatment. The greatest inhibition of total HAA formation was achieved with diallyl disulfide (DAD) (78%) and dipropyl disulfide (DPD) (70%). These compounds also significantly ($p < 0.05$) reduced overall mutagenicity, with reductions of 75 and 65% for DAD and DPD, respectively. The addition of diallyl sulfide, allyl methyl sulfide, and allyl mercaptan also significantly ($p < 0.05$) reduced mutagenicity, with reductions of 56, 43, and 30% being noted, respectively. The addition of cysteine and cystine, however, did not reduce the mutagenicity of cooked meat, an observation confirmed by the relatively small reductions in HAA concentrations. These results suggested that the addition of selected sulfur compounds to ground beef may be an alternative approach to reduce HAA formation and overall mutagenicity of cooked beef patties.

INTRODUCTION

Epidemiological studies have shown that diet and life style are closely related to human cancer (Sugimura and Sato, 1982). Many mutagens and carcinogens have been identified in foods. Recently, several foods and constituents of foods have been investigated for their inhibitory or promotional effects on carcinogenesis (Knize et al., 1999; Weisburger, 1991).

Heterocyclic aromatic amines (HAAs) are produced in muscle foods during cooking, and many have been shown to be mutagenic and/or carcinogenic. These compounds have been classified into two categories, pyrolytic mutagens and thermic mutagens. Pyrolytic mutagens are formed when proteins, amino acids or proteinaceous food are heated to high temperatures (>300 °C) and are characterized by a pyridine ring with an amino group attached (Skog, 1993; Wakabayashi and Sugimura, 1998). Thermic mutagens are formed at lower temperatures (<300 °C) and several have been identified in cooked fish and meat products. These compounds, also called aminoimidazoazaarenes, have been characterized as quinolines, quinoxalines, pyridines or furopyridines. The most commonly found HAAs in foods are IQ (2-amino-3-methylimidazo[4,5-*f*]-quinoline); MeIQ (2-amino-3,4dimethylimidazo[4,5-*f*]-quinoline); MeIQx (2-amino-3,8 dimethylimidazo [4,5-*f*]-quinoxaline); 4,8 DiMeIQx (2-amino-3,4,8 trimethylimidazo[4,5-*f*]-quinoxaline); and PhIP (2-amino-1-methyl-6-phenylimidazo [4,5-*b*]-pyridine) (Knize et al., 1999; Skog, 1993; Wakabayashi and Sugimura, 1998).

Garlic has been cultivated since antiquity and has been used as foodstuffs and medicines. In recent years, garlic has become highly valued because of its excellent

effectiveness on arteriosclerosis, its ability to lower serum cholesterol and triglyceride levels, and for its hypotensive, anticarcinogenic and antidiabetic effects. Garlic also inhibits thrombocyte aggregation and activates fibrinolysis (Kabelik, 1970; Kendler, 1987; Kritchevsky, 1991; Lutomski, 1983; Reuter and Sendl, 1994; Weiss, 1986).

Food preparation methods have a significant influence on HAA formation and much research has been devoted to the carcinogens in fried and broiled food. Food preparation methods in many homes include the cooking of meats with various vegetables containing naturally occurring organosulfur compounds. In the previous chapter, it was demonstrated that garlic and several endogenous organosulfur compounds, when added to ground beef patties before frying, will inhibit or greatly reduce HAA formation. However, there is a need to ascertain if there is a concomitant reduction in overall mutagenicity of the fried beef with the reduced concentrations of HAAs. This need is based on the proposition that organosulfur compounds could react with meat components during cooking to generate other mutagenic species. Therefore, the objective of this study was to characterize the relationship, if any, between the reduction of total HAA concentrations and overall mutagenicity of fried ground beef patties to which were added selected organosulfur compounds before frying.

MATERIALS AND METHODS

Safety

Heterocyclic aromatic amines are mutagenic and/or carcinogenic and should be handled with appropriate safety precautions, including the use of goggles, latex gloves and efficient fume hoods.

Materials

Diallyl disulfide (DAD), dipropyl disulfide (DPD), diallyl sulfide (DAS), allyl methyl sulfide (AMS), allyl mercaptan (AM), cysteine, cystine, and dimethyl sulfoxide (DMSO) were purchased from Fluka Chemical Co. (Buchs, Switzerland). The HAA standards (MeIQx, 4,8-DiMeIQx, and PhIP) were obtained from Toronto Research Chemicals (Toronto, Canada). The HAA standard (FEMA-Flavor and Extracts Manufacturer's Association) and the internal standard, caffeine, were gifts from Dr. Mark Knize, Lawrence Livermore National Laboratory, Livermore, CA. The FEMA standard contained IQ, MeIQ, MeIQx, DiMeIQs, and PhIP, each at 5 ng/μL. Propyl-sulfonic acid (PRS) Bond-Elut columns (500 mg) and C18 (100 mg) cartridges were purchased from Varian Inc. (Harbor City, CA). Extrelut-20 columns and Extrelut diatomaceous earth were obtained from E.M. Separations Technology (Gibbstown, NJ). All other chemicals were of analytical grade and were purchased from Fisher Scientific (Fair Lawn, NJ).

Freshly ground beef was obtained from a local supermarket and used within one hour of purchase, or stored at -20°C until required for frying. The fat content was determined by the method of Folch et al. (1957).

Preparation of ground beef patties

Patties were prepared from ground beef as follows: control patties (ground beef patties mixed with 1 mL methanol) and patties containing organosulfur compounds added as separate treatments two hours before frying. Organosulfur compounds (DAD, DPD, DAS, AMS, AM, cysteine, and cystine) at a concentration of 0.67 mmol were dissolved in 1 mL methanol, added directly to the ground beef, and mixed in a Keebler mixer (Keebler Inc., Chicage, IL) for 2 min. Each patty (100g) was formed by placing the ground beef in a petri dish (9 cm dia. × 1.5 cm thickness) to ensure patty uniformity. The study was repeated three to five times using one batch of ground beef with a fat content of 15.4±2.0%.

Cooking of patties

Patties were fried in a teflon-coated electric frying pan at 225°C (surface temperature) for 10 min per side. The temperature of the frying was determined using a surface temperature thermometer (Pacific Transducer Corp., Los Angeles, CA). Two patties were fried for each replication, and five replicates were analyzed for each treatment. For each replicate, four sub-samples were analyzed (two unspiked for concentration and two spiked for recovery). The cooked meat patties were mixed in a blender to produce a uniform sample and frozen at -4°C until extraction.

Extraction of HAAs from meat samples

The HAAs were extracted from the meat samples and purified using solid-phase chromatography following the standard addition procedure of Gross and Grüter (1992).

Meat samples were extracted by homogenizing 30 g cooked meat with 90 g 1N NaOH. The homogenate was divided into four equal aliquots. To determine extraction recoveries, two of the aliquots were spiked with 250 ng of each of the following HAAs (IQ, MeIQ, MeIQx, DiMeIQx and PhIP) dissolved in 50 μl methanol. Samples were mixed with Extrelut diatomaceous earth (Varian, Inc, Harbor city, CA) to fill an Extrelut 20 column. All four extractions were made with 40 mL dichloromethane containing 5% toluene (v/v) using attached Bond Elut PRS extraction columns. One unspiked aliquot from each meat sample was processed for the Ames/Salmonella assay as indicated below. For HAA analyses, the PRS cartridges were washed with 6mL 0.1N HCl, 15 mL 40% methanol in 0.1N HCl, and followed by 2ml water. The HAAs were transferred to Bond Elut C18 cartridges (100mg) with 20 mL ammonium acetate buffer (0.5 M, pH8.0). The cartridges were eluted with 0.8 mL MeOH:NH_4OH (9:1, v/v). The elutes were evaporated to dryness and dissolved in 50 μl methanol containing 5 ng/ul caffeine as an internal standard. For mutagenic activity testing which does not require further sample clean-up, the remaining aliquot was eluted from the PRS cartridge with 2.0 ml MeOH-NH_4OH, evaporated to dryness and dissolved in 120 μL dimethyl sulfoxide (DMSO).

HPLC analyses

HAA analyses were performed by high pressure liquid chromatography (HPLC) as described in Chapter 2.

Salmonella mutagenicity assay

The mutagenic activity of the sample extracts was determined using the standard plate incorporation assay described by Ames et al. (1975) using *Salmonella typhimurium* TA98 (Molecular Toxicology, Inc; Boone, NC). Aroclor-induced rat liver S-9 mixture (Molecular Toxicology, Inc) was used for metabolic activation. DMSO was used as a negative control (spontaneous revertant colonies), while 2-aminoanthracene was used as a positive control for *S. typhimurium* TA98. The latter gave an average of 850 revertants/μg. To determine calculated values of revertants/g meat, individual HAA standards (MeIQx, DiMeIQx, and PhIP) were tested under similar conditions. The concentrations of HAAs obtained by HPLC analyses were then multiplied by these values to determine the calculated overall mutagenicity. Mutagenic activity was calculated from the linear portion of the dose-response curve using the method of Moore and Felton (1983). A minimum of four dose points from duplicate platings were used, and the linear portion of the curves was used to calculate the revertants/g cooked beef patties.

Statistical analyses

The results were analyzed by Sigma Stat 2.0 (Jandel Corp., San Rafael, CA). One-way analysis of variance (ANOVA) was performed. Appropriate comparisons were made using Student-Newman-Keuls test for one-way ANOVA analysis. Calculation of mutagenic activity was made by linear regression analysis of the dose response curves of revertants/μg of HAAs or g of cooked beef patties (Neter et al., 1993).

RESULTS AND DISCUSSION

Reduction of HAA formation and overall mutagenicity in ground beef patties by organosulfur compounds

Ground beef patties were fried at 225°C for 10 min/side to evaluate the effect of organosulfur compounds on HAA formation. Confirming what was established in Chapter 2, various organosulfur compounds, when added directly to ground beef before frying, inhibited HAA formation to varying degrees (Table 1). The addition of DAD and DPD to ground beef patties inhibited total HAA formation by 78 and 70%, respectively. Reductions in PhIP concentrations were 82 and 73%, respectively. These compounds also inhibited DiMeIQx formation in ground beef patties by 82% (DAD) and 69.9% (DPD). The amount of inhibition of HAA formation by DAD was higher than all the other organosulfur compounds (DPD, DAS, AMS, and AM). These results confirm those reported in Chapter 2, while the DAD data support the results of Tsai et al. (1996) who demonstrated that DAD was an effective inhibitor of mutagen formation in boiled pork juice.

Several other literature reports allude to the reduction of mutagens in meat products through the addition of onion or garlic extracts. Reduction in mutagen formation in cooked ground beef with onion juice has been reported by Kato et al. (1998). Murkovic et al. (1998) reported that garlic reduced PhIP concentrations in fried ground beef by 78%, although these investigators did not specifically address the mode of action involved, or measure the impact of HAA reduction on overall mutagenicity. The results presented here clearly confirm selected organosulfur compounds as effective

Table 1. Effect of various sulfur compounds on the formation of heterocyclic aromatic amines in ground beef patties[1,2]

	Heterocyclic aromatic amines (ng/g)[3]			
Treatment	MeIQx	DiMeIQx	PhIP	Total HAAs
Control	6.1±0.8[a]	2.8±0.5[a]	15.7±2.1[a]	24.6
DAD	2.0±0.2[c]	0.5±0.1[d]	2.9±0.6[d]	5.4
Inhibition (%)	68	82	82	78
DAS	3.1±0.3[b,c]	1.3±0.2[b,c]	6.9±0.9[c]	11.2
Inhibition (%)	50	54	56	55
AMS	3.6±0.3[b]	1.8±0.3[b]	8.6±1.4[b]	13.9
Inhibition (%)	38	37	45	43
AM	3.9±0.4[a,b]	1.9±0.3[b]	10.1±1.5[b]	15.8
Inhibition (%)	34	34	36	36
DPD	2.3±0.6[c]	0.9±0.2[d]	4.2±0.9[d]	7.4
Inhibition (%)	62	70	73	70
Cystine	4.4±0.6[a]	2.1±0.4[a]	11.4±1.5[a]	17.9
Inhibition (%)	28	26	27	27
Cysteine	5.0±0.5[a]	2.4±0.5[a]	12.8±1.8[a]	20.2
Inhibition (%)	19	14	18	17

[1] Values are based on measured cooking losses for individual patties.
[2] Sulfur compounds were added at the 0.67 mmol concentration to ground beef patties.
[3] Means with the same superscript are not significantly different ($p>0.05$).
Data are the means of five replicates.

inhibitors of HAA formation in ground beef patties, although at the concentrations used they would probably impart an unacceptable flavor to the beef patties.

The effect of these organosulfur compounds on the overall mutagenicity of cooked beef patties was evaluated by the Ames *S. typhimurium* assay using the tester strain TA98 (Figure 1). DAD and DPD significantly ($p < 0.05$) reduced mutagenicity by 75 and 65%, respectively, with the number of revertants being lowered from 905 revertants/g of meat to 226 and 321 revertants/g of meat, respectively. The addition of DAS, AMS, and AM also significantly ($p < 0.05$) reduced mutagenicity, with reductions of 56, 43, and 30%, respectively, being obtained. However, cysteine and cystine did not significantly reduce mutagenicity of the cooked meat patties. These results are comparable to those reported by Tsai et al. (1996) who demonstrated a 80-98% reduction in overall mutagenicity in boiled pork juice through the addition of DAD and DPD. They also reported no significant impact of cysteine and cystine on overall mutagenicity of the boiled pork jucie model system. Trompeta and O'Brien (1998) also demonstrated the mutagenicity-lowering activity of various sulfur compounds in a model system containing glucose, glycine, and creatinine.

Measured and calculated mutagenicity in fried ground beef patties

The *S. typhimurium* mutagenicity test is desirable when assessing the evaluation of overall mutagenicity by the reduction of HAA concentrations in meat products with specific food ingredients. There is a possibility that other mutagenic compounds may be introduced into the fried ground beef through the interaction of the potential inhibitor with meat components, or by the thermal breakdown of the inhibitor itself. The results

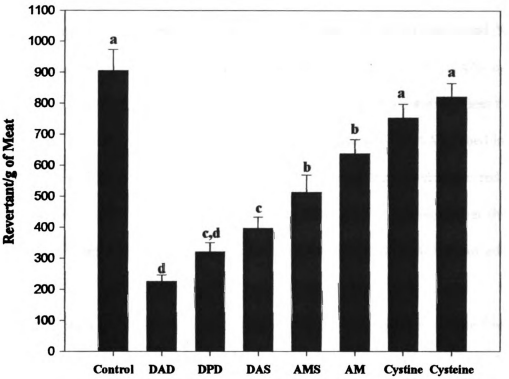


Figure 1. The effect of organosulfur compounds on the mutagenicity of ground beef patties as determined by the Ames *S. typhimurium* TA98 assay. Bars with different letters are significantly different ($p<0.05$). All treatments were replicated five times.

presented here indicate that the inhibition of HAA formation in fried ground beef patties by organosulfur compounds, as measured by HPLC analyses, is accompanied by a concomitant reduction in the overall mutagenicity of the patties.

The mutagenic activity of each HAA standard was determined by the *S. typhimurium* TA 98. The mutagenic activity of MeIQx, DiMeIQx, and PhIP standard determined by the *S. typhimurium* TA 98 were 82900, 19800, and 1900 revertants/μg of HAA, respectively. These results agree with the data of Wakabayashi and Sugimura (1998), who demonstrated that MeIQ is the most mutagenic of the HAAs evaluated, followed by IQ, MeIQx, DiMeIQx, and PhIP. Although PhIP contributes less than 18% of the total mutagenic activity of meat, it is the most abundant HAA formed in cooked meat (Skog et al., 1995). Therefore, it would be expected that a significant reduction of the mutagenicity of cooked beef patties in this study would also mean that there were meaningful reductions in the concentrations of MeIQx, and DiMeIQx, in addition to PhIP.

We were interested in correlating the measured mutagenicity of the fried patties by the *S. typhimurium* assay with mutagenicity values calculated from the concentrations of the HAAs in the fried ground beef that were quantified by HPLC. The Ames assay may determine mutagenic activity not totally accounted for by HAA concentrations. The plot of measured and calculated mutagenicity is shown in Figure 2. The measured mutagenicity in each sample was quite similar to the mutagenicity values calculated from the determined concentrations of HAAs. These observations agree with those of Felton et al. (1994) who reported that measured mutagenicity in fried beef patties was similar to the mutagenicity calculated from the measured concentrations of

HAAs. The linear regression between measured and calculated activities (slope 0.88, $R^2 = 0.96$) indicates that the concentrations of the determined HAAs are responsible for most, but not all, of the mutagenicity detected. Other HAAs such as IQ, IQx, and MeIQ are found infrequently in cooked beef, but when present, are there in very low amounts. Felton and Knize (1990) reported that PhIP in fried ground beef accounted for 86-91% of the total mass of the mutagenic compounds. MeIQx and DiMeIQx contributed 9.4-11.7% of the total mass, while IQ, IQx, and MeIQ were only found in very small concentrations.

The results also demonstrated that the addition of sulfur compounds did not result in the formation of other mutagenic compounds in the ground beef through the interaction of the potential inhibitor with meat components, or by the thermal breakdown of the inhibitor itself. The scatter in the data is probably due to a combination of measurement errors in solid-phase extraction procedures and the accumulation of errors in each analytical method. Knize et al. (1994) and Turesky et al. (1988) reported that about 80% of the mutagenicity could be accounted for by quantitative HPLC analyses of HAAs in cooked beef and that the *S. typhimurium* mutagenicity assay would be a reasonable screening method to determine HAA formation in cooked meat samples. On the basis of the results of the present study, the latter procedure could effectively evaluate the effects of selected food ingredients on HAA formation in fried ground beef patties.

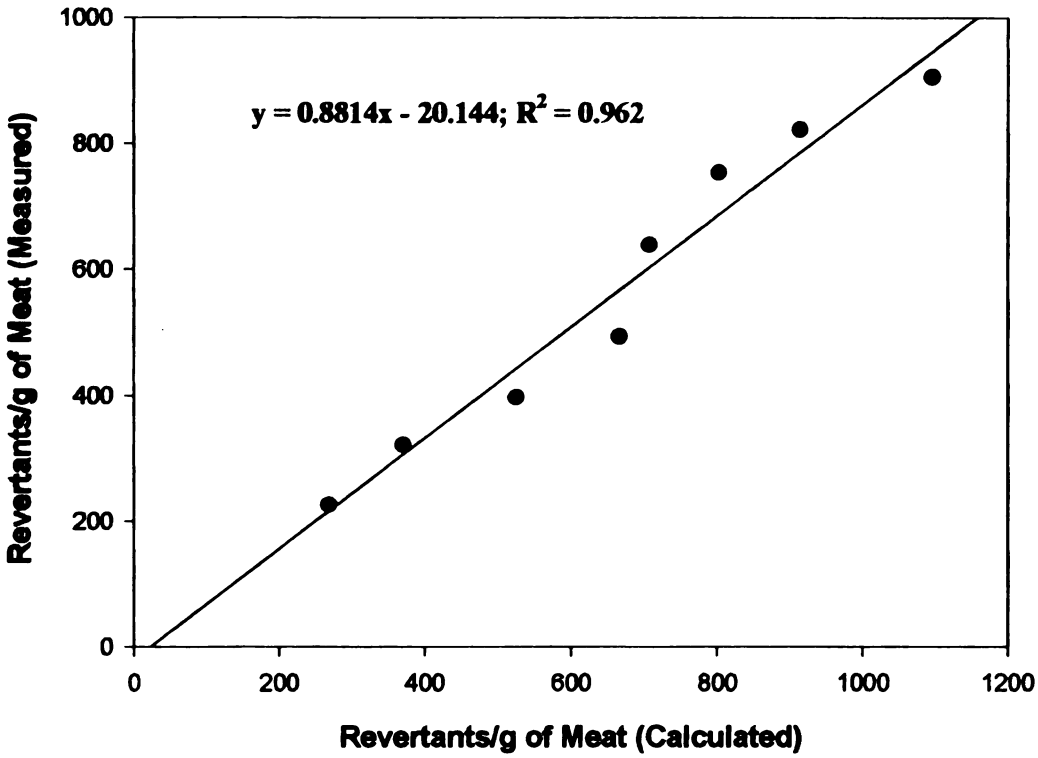


Figure 2. Plot of mutagenic activity quantitated by the *S. typhimurium* TA98 assay and mutagenic activity calculated from the heterocyclic aromatic amine concentrations in fried beef patties as determined by HPLC analyses. Each data point is the mean of five replicated experiments.

CHAPTER FOUR

A model system study of the inhibition of heterocyclic aromatic amine formation by organosulfur compounds

ABSTRACT

Organosulfur compounds and sodium bisulfite significantly inhibited ($p<0.05$) heterocyclic aromatic amine (HAA) formation in model systems containing phenylalanine, creatinine, and glucose. However, there was no inhibition by the same compounds in a model system containing only phenylalanine and creatinine. Diallyl disulfide (DAD) and dipropyl disulfide (DPD) concentrations in the model system significantly decreased ($p<0.05$) after heating for 10 min at 180°C. These decreases could be through their interaction with glucose and/or by their decomposition. Only very low concentrations of sulfhydryl groups (4.19 μmol and 4.00 μmol) were formed on heating DAD and DPD for 30 min. Reaction of glucose and DAD produced several sulfur-containing compounds. After 10 min of heating at 180°C, HAA formation in the control model systems was increased significantly, while DAD was an effective inhibitor during this heating period. Tetrahydrothiophene-3-one (THT) and tetrahydrothiophene (THP); two products assessing from the interaction of glucose and DAD, had no direct influence on HAA formation in the model systems. These observations indicate that DAD and DPD may function as HAA inhibitors by their ability to react with glucose, thus possibly reducing the latter's availability to react with phenylalanine.

INTRODUCTION

When food is cooked, carbonyl and amino compounds react via what is known as the Maillard reaction (Hodge, 1953). Several hundreds of reaction products are produced, some of which contribute to the color and flavor of the cooked foods. The Maillard reaction may also impact the nutritional value of the food (Reynolds, 1963). Furthermore, in some cases, the Maillard reaction can lead to the formation of genotoxic compounds called heterocyclic aromatic amines (Powrie et al., 1981; Shibamoto et al., 1981; Spingarn and Garvie, 1979; Wei et al., 1981).

Heterocyclic aromatic amines (HAAs) are formed in cooked meat and fish products, most likely from the reaction of proteins, sugar and creatine (Skog, 1993). These compounds have been shown also to induce colon, breast, pancreas, and prostate cancer (Stavric, 1994). The most commonly found HAAs in foods are IQ (2-amino-3-methylimidazo[4,5-*f*]-quinoline); MeIQ (2-amino-3,4dimethylimidazo [4,5-*f*]-quinoline); MeIQx (2-amino-3,8 dimethylimidazo [4,5-*f*]-quinoxaline); 4,8 DiMeIQx (2-amino-3,4,8 trimethylimidazo[4,5-*f*]-quinoxaline); and PhIP (2-amino-1-methyl-6-phenylimidazo [4,5-*b*]-pyridine) (Knize et al., 1999; Skog et al., 1992; Wakabayashi and Sugimura, 1998). PhIP shows weak mutagenic activity in the Ames *Salmonella* assay, but is the most abundant mutagen present in cooked food (Stavric, 1994).

It has been postulated that pyrazines/pyridines, aldehydes, and creatine/creatinine are condensed to form IQx- and IQ-type compounds (Jägerstad et al., 1983a; Milic et al., 1993). It has also been determined that PhIP is formed from the reaction of creatinine and glucose with certain amino acids such as phenylalanine, isoleucine, or tyrosine (Johansson and Jägerstad, 1993). Factors that influence the

formation of HAAs in foods include precursor concentrations, type of amino acids, and cooking time and temperature (Skog, 1993). Certain food ingredients can reduce HAA formation in foods such as vitamin E (Balogh et al., 2000), cherry tissue (Britt, 1998), tea polyphenolic compounds (Yen and Chen, 1995), soy protein concentrate (Wang et al., 1982) and defatted glandless cottonseed flour (Rhee et al., 1987).

Garlic is a commonly used foodstuff. In addition, a variety of garlic-based health products are now readily available on the market. Most of their health promoting claims are based on the presence of organosulfur compounds such as allicin, diallyl disulfide (DAD), diallyl sulfide (DAS), and dipropyl disulfide (DPD). DAD, for example, has been shown to have a positive effect on arteriosclerosis (Kritchevsky, 1991), and on serum cholesterol (Reuter and Sendl, 1994) and triglyceride levels (Weiss, 1986). In addition, it has exhibited hypotensive (Kendler, 1987), anticarcinogenic (Wattenberg et al., 1989) and antidiabetic effects (Augusti and Sheela, 1996).

It has been established that organosulfur compounds will inhibit Maillard browning reactions (Friedman, 1996; Trompeta and O'Brien, 1998). The inhibition of this reaction may be the key to reducing HAA formation in foods through the addition of garlic and other organosulfur compounds. However, the mechanism by which organosulfur compounds inhibit the Maillard reaction has not been fully elucidated.

We have established that organosulfur compounds, when added to ground beef patties before frying, will inhibit or greatly reduce HAA formation and overall mutagenicity (Chapters 2 and 3). However, the mechanism by which HAA formation is influenced by organosulfur compounds is unclear. The objective of this study is to

better understand how the formation of HAAs is inhibited by the addition of sulfur compounds to food and model systems.

MATERIALS AND METHODS

Safety

HAAs are mutagenic/carcinogenic and should be handled with appropriate safety precautions including the use of goggles, latex gloves and efficient fume hoods.

Materials

Phenylalanine, glycine, glucose, creatinine, sodium bisulfite, cysteine, DTNB (5,5'-dithio-bis-(2-nitrobenzoate), tridecane, Tris-HCl, tetrahydrothiophene-3-one (THT), and teetrahydrothiophene (THP) were purchased from Sigma Chemical Company (St. Louis, MO). DAD, DPD, and allyl mercaptan (AM) were purchased from Fluka Chemical Co. (Buchs, Switzerland). The HAA standards (IQx, MeIQx, and PhIP) were obtained from Toronto Research Chemicals (Toronto, Canada). The HAA standard (FEMA-Flavor and Extracts Manufacturer's Association) and the internal standard, caffeine, were gifts from Dr. Mark Knize, Lawrence Livermore National Laboratory, Livermore, CA. The FEMA standard contained IQ, MeIQ, MeIQx, DiMeIQs, and PhIP, each at 5 ng/μL. Propyl-sulfonic acid (PRS) Bond-Elut columns (500 mg) and C18 (100 mg) cartridges were purchased from Varian Inc. (Harbor City, CA). Extrelut-20 columns and Extrelut diatomaceous earth were obtained from E.M.

Separations Technology (Gibbstown, NJ). All other chemicals were of analytical grade and were obtained from Fisher Scientific (Fair Lawn, NJ).

The heating module was a Reacti-Therm III, model 18835, made by Pierce Co. (Rockford, IL). Stainless steel test tubes, 2.3 ml capacity, with threaded, self-sealing stainless steel caps were manufactured by the Enginering Research Complex Machine Shop at Michigan State University. A new set stainless steel test tube was used for each amino acid to avoid carryover of HAAs from one experiment to another.

Effect of organosulfur compounds and sodium bisulfite on HAA formation in model systems

The control model system contained 0.6 mmol phenylalanine and 0.6 mmol creatinine in 1.5 mL water, with or without 0.3 mmol glucose. The reactants were added directly to the stainless test tubes and sealed with threaded caps wrapped with Teflon tape. Organosulfur compounds (0.67 mmol DAD, DPD, AM, and cysteine) and sodium bisulfite (0.67 mmol) were added to the model systems. The reaction was carried out in a closed hood separated from the rest of the laboratory. The Reacti-Therm heating module was allowed to preheat for a minimum of 1.5 hr before heating the samples. The heating temperature was 180° ± 5°C and silicon oil (0.5 ml) was placed in each cavity in the heating block to facilitate heat transfer from the block to the test tubes. The stainless test tubes were heated for 30 min and then immediately cooled in an ice-bath. The contents of each test tube was quantitatively transferred to microvials (1.5 ml capacity) and stored at 5° ± 1°C until required.

The contents of two 1.5 ml microvials were mixed with 57 ml 5N NaOH and stirred with a spatula. Aliquots (10 ml) were taken from the mixture and placed in four 250 ml beakers. The remainder of the mixture was saved and used if further extractions were required. To determine extraction recoveries, two of the aliquots were spiked with 1.0 μg each of IQx, MeIQx, and PhIP dissolved in 50 μl methanol. The rest of the extraction, purification, and quantitation procedures were similar to those described in Chapter 2.

Effect of heating on the stability of organosulfur compounds

Organosulfur compounds (0.67 mmol DAD or DPD), with or without model system reactants (0.3 mmol glucose, 0.6 mmol phenylalanine and 0.6 mmol creatinine), were heated at 180°C for 30 min as described previously. Samples were taken at 10 min intervals to quantitate DAD and DPD concentrations remaining in the model system. A HP 5890 gas chromatograph (Hewlett Packard, Avondale, PA), equipped with a fused silica capillary column (60 m × 0.25 mm i.d.; 1 μm thickness, DB-1, J&W Scientific Inc., Folsom, CA) and a flame ionization detector, was used to determine the DAD and DPD concentrations. The analytical conditions were as follows: injector temperature, 270°C; detector temperature, 300°C; helium carrier flow rate, 1 ml/min; temperature program, 40°C (5 min), 2°C/min, 260°C (60 min). A split ratio of 50:1 was used. DAD and DPD concentrations were determined using the peak areas on the gas chromatograms. The peak area of DAD or DPD from the control system before heating was standardized as 100%, and DAD or DPD concentrations from the reaction mixture were calculated.

Effect of heating on formation of sulfhydryl groups

Each sample contained 0.67 mmol DAD or DPD in 1.5 ml water. These samples were mixed directly in the stainless test tubes and heated at 180° ± 5°C for 30 min as described previously. An aliquot (0.1 ml) of the heated sample was diluted with 0.02 ml 10 mM DTNB (5,5'-dithio-bis-(2-nitrobenzoate), 0.1 ml 0.2 M Tris-HCl buffer (pH 7.5), and 0.78 ml water and its turbidity was measured at 412 nm using a UV spectrophotometer (Varian Inc., Harbor City, CA). Sulfhydryl concentrations (μmoles in 1.5mL) were calculated using the Beer-Lambert law equation:

$$\Delta A = \Delta a_m \cdot c \cdot b$$

where ΔA is the absorbance at 412 nm, Δa_m is the extinction coefficient (13,600 M^{-1} cm^{-1}), c is the concentration, and b is the sample thickness (1 cm).

Formation of volatile compounds in heated model systems

Samples containing 0.67 mmol DAD (or 0.3 mmol glucose, or 0.67 mmol DAD and 0.3 mmol glucose) in 1.5 ml water were heated in stainless steel test tubes at 180° ± 5°C for 30 min. After cooling rapidly in an ice bath, the reaction mixtures were mixed with an internal standard (tridecane, 100 μl) and extracted four times with 10 ml dichloromethane. The combined extracts were dried over anhydrous sodium sulfate and concentrated to 5 ml in a Kuderna-Danish concentrator (Supelco, Bellefonte, PA). The extract was then concentrated to 1 ml under nitrogen. The generated volatile compounds were separated by gas chromatography using the same conditions as described in the previous section.

The concentrated isolates were analyzed by GC-mass spectrometry (MS) using a HP 5890 gas chromatograph interfaced with a HP 5970 MSD (mass selective detector) mass spectrometer. The GC/MS system was equipped with a HP 59970 Chemstation Data System. The GC was operated under the same conditions as previously described. The MS was operated in the electron impact mode with an electron energy of 70 eV and an ion source temperature of 250°C. Compounds were introduced to the ion source directly from the capillary column in the GC using an open-split interface. A continuous scan mode with a scan time of 1 sec over a mass range of 40-300 was employed. The GC/MS data were monitored, stored and analyzed using a HP Chemstation data system. Several compounds in the isolate were identified by comparing their mass spectral data with those of authentic compounds available in the NIST/EPA/MSDC Mass Spectral Database purchased from ACS Publication Co. (Washington, DC) or INRA MassSpectra Computer Library (Laboratoire de Recherche sue les Aromes, Dijon, France).

Effect of heating time on HAA formation and inhibition in model systems containing DAD or DPD

Several model systems were evaluated. The control system contained 0.6 mmol phenylalanine, 0.6 mmol creatinine, and 0.3 mmol glucose in 1.5 ml water. Other systems contained 0.67 mmol DAD (or DPD) in addition to the three primary reactants. Samples were heated for 30 min at 180°C as described previously, with 3 ml aliquots being taken at 10 min intervals. The effect of the sulfur compounds on HAA formation

was determined by extraction, purification, HPLC analysis, and HAA peak identification as described earlier.

Effect of tetrahydrothiphene-3-one (THT) and tetrahydrothiophene (THP) on HAA formation in various model systems

The direct effect of THT and THP on HAA formation in various model systems was evaluated by adding (0.67 mmol THT of THP) to model systems containing 0.6 mmol creatinine, 0.6 mmol phenylalanine or glycine, and with or without 0.3 mmol glucose in 1.5 ml water. The reactants were heated at 180°C for 30 min. Samples were extracted and purified as previously described and analyzed by HPLC.

Statistical Analysis

The results were analyzed by Sigma Stat 2.0 (Jandel Corp., San Rafael, CA). One way analysis of variance (ANOVA) was performed for each HAA. Appropriate comparisons were made using Student-Newman-Keuls test for one way ANOVA analysis.

RESULTS AND DISCUSSION

Effect of organosulfur compounds and sodium bisulfite on HAA formation in model systems

The major HAAs detected in the heated model systems containing glucose, phenylalanine, and creatinine were IQx, MeIQx, and PhIP (Table 1). As expected, the dominant HAA was PhIP (34.4±8.37 nmol/mmol creatinine), followed by MeIQx (12.9±6.98 nmol/mmol creatinine) and IQx (7.2±4.17 nmol/mmol creatinine). These observations agree with those of Shioya et al. (1987) and Skog and Jägerstad (1991). The average recoveries of HAAs from the model system were 87±16, 85±18, and 82±15% for IQx, MeIQx, and PhIP from similar model systems, respectively. These recoveries are comparable to those of Scranton (1997) who reported average recoveries of 88, 88, and 71% for IQx, MeIQx, and PhIP, respectively. Phenylalanine is the major amino acid contributing to PhIP formation in meat and model systems (Shioya et al. 1987; Skog and Jägerstad, 1991). Concentrations of PhIP in beef have been estimated to be ten times greater than those of the other known HAAs combined (Felton et al., 1986b). Therefore, it may play an important role in the etiology of cancer, even though PhIP is less mutagenic than other HAAs (Stavric, 1994).

Concentrations of PhIP were significantly reduced ($p < 0.05$) upon the addition of organosulfur compounds to the model systems before heating. The percentage inhibition ranged from 73% for DAD to 42% for AM. MeIQx concentrations were reduced 82% by DAD and 63% by DPD. Cysteine did not significantly reduce HAA

Table 1. Effect of organosulfur compounds and sodium bisulfite on the formation of heterocyclic aromatic amines in a model system containing phenylalanine, creatinine, and glucose[1]

	Heterocyclic aromatic amines (nmol/mmol of creatinine)		
Treatment	IQx	MeIQx	PhIP
Control	7.2±4.2[a]	12.9±3.0[a]	34.4±8.4[a]
DAD	1.8±1.1[b]	2.2±0.7[c]	8.2±4.2[c]
Inhibition (%)	75	82	73
DPD	2.9±1.8[b]	4.9±1.9[b]	12.6±3.7[c]
Inhibition (%)	61	63	56
Bisulfite	1.7±0.4[b]	2.1±0.7[c]	7.8±2.0[c]
Inhibition (%)	77	84	78
AM	4.4±1.5[a]	7.5±2.2[a]	19.9±4.1[b]
Inhibition (%)	39	42	42
Cysteine	5.6±2.2[a]	10.2±3.4[a]	29.5±6.5[a]
Inhibition (%)	22	20	14

[a-c]Means with different superscripts are significantly different ($p<0.05$). Comparisons are made only within the same column. Means±standard deviations; n=5 for all treatments. [1]Heated at 180°C for 30 min.

formation, an observation similar to that described previously for fried ground beef patties (Chapter 2).

Sodium bisulfite also significantly ($p < 0.05$) inhibited HAA formation, with reductions of 77, 84, and 78% being achieved for IQx, MeIQx, and PhIP, respectively. The inhibitory effect of sodium bisulfite on HAA formation has been reported previously by Krone and Iwaoka (1987), who observed a reduction in the mutagenicity of canned salmon upon the addition of bisulfite. Chen (1988) also demonstrated sodium bisulfite inhibition of IQx, MeIQx, and DiMeIQx formation in fried ground beef patties, although he did not offer an explanation for its inhibitory action.

The inhibition of the Maillard reaction by sodium bisulfite has been established through its interaction with reducing sugars (Wedzicha, 1992). Several chemical mechanisms have been proposed to explain its inhibitory action. One of the most important involves its addition to the carbonyl group of reducing sugars and other carbonyl compounds participating in the Maillard reaction (Wedzicha, 1992). The reaction between sodium bisulfite and the carbonyl group of reducing sugars produces a 3,4-dideoxy-4-sulfohexosulose, a result of nucleophilic attack by the sulfite ion on the α,β-unsaturated carbonyl moiety of 3,4-dideoxyhexosulos-3-ene. This reaction may compete with pathways that are involved in further Maillard reactions (Wedzicha, 1992).

It has been speculated that HAA formation occurs through intermediates of the Maillard reaction. Jägerstad et al. (1983a) proposed that pyridines and pyrazines, formed via the Maillard reaction, react with an aldehyde to form a quinoline or quinoxaline. Such structures are integral parts of the HAA molecule. Creatine

undergoes dehydration and cyclization to form creatinine when heated, which then reacts with an aldehyde to form an IQ- or IQx-type HAA. Inhibition of the Maillard reaction by sodium bisulfite thus appears to be a likely mechanism by which HAA formation is inhibited/reduced in those systems containing bisulfite.

To further establish that the inhibitory action of sodium bisulfite, and possibly that of the organosulfur compounds under evaluation, is via reaction with glucose, a study was designed to investigate HAA formation in model systems containing phenylalanine and creatinine (i.e., sugar-free). The only HAA detected in this system was PhIP (Table 2). These results agree with those of Overvik et al. (1989) and Skog and Jägerstad (1991). The latter investigators further demonstrated that the yield of PhIP increased three-fold when glucose was added to the reaction mixture and heated under similar conditions. Furthermore, small concentrations of the IQ-type HAAs were present in the model systems. Our results (Tables 1 and 2) confirmed these observations, both with respect to the effect of glucose on HAA formation (3-4 fold increases in PhIP concentrations) and to the quantities of the other HAAs produced.

Sodium bisulfite and organosulfur compounds did not inhibit PhIP formation in the model systems that did not contain glucose (Table 2). Because sodium bisulfite reacts directly with glucose, it may be inferred that HAA inhibition by DAD and DPD in model systems containing glucose could also be through their interaction with glucose. Understanding how this occurs is the basis of the next series of experiments.

Effect of heating on the stability of organosulfur compounds

Table 2. Effect of organosulfur compounds and sodium bisulfite on the formation of heterocyclic aromatic amines in a model system containing phenylalanine and creatinine[1]

	Heterocyclic aromatic amines (nmol/mmol of creatinine)		
Treatment	IQx	MeIQx	PhIP
Control	ND	ND	10.2±2.2[a]
DAD	ND	ND	10.5±1.5[a]
DPD	ND	ND	11.1±1.7[a]
Bisulfite	ND	ND	10.2±1.7[a]

[a-b]Means with different superscripts are significantly different ($p<0.05$). Comparisons are made only within the same column. Means±standard deviations; n=5 for all treatments.
ND = Not detectable. Limit of detection is 0.4 ng.
[1]Heated at 180°C for 30 min.

In order to determine whether DAD or DPD undergoes decomposition in the model systems, a study was designed to quantitate DAD or DPD concentrations after 10, 20, and 30 min of heating at 180°C. Figure 1 shows that DAD and DPD concentrations in the model system containing glucose, creatinine, and phenylalanine decreased by 57 and 46%, respectively, after only 10 min of heating at 180°C. These decreases may be due to their interaction with components of the model system and/or by their own thermal decomposition. When DAD (or DPD) was heated alone under similar conditions (30 min at 180°C), markedly smaller decreases in concentration (24-43%) were observed (Figure 1). These decreases may be attributed to decomposition of the sulfur compounds or by their vaporization from the sealed tubes. The differences in the percentage losses of DAD and DPD in the various model systems may be explained by their interaction with other model system reagents, namely glucose, creatinine, and phenylalanine.

A possible mode of loss of the disulfide compounds is through dissociation to sulfhydryl compounds on heating. This time of inquiry is important because Friedman and Molnar-Perl (1990) proposed that sulfhydryl groups inhibit the Maillard reaction through their interaction with intermediates formed during the Maillard reaction and by suppression of free-radical formation. Heating DAD and DPD at 180°C for 30 min produced low concentrations of sulfhydryl compounds, 4.19 and 4.00 μmol being produced from heating DAD and DPD, respectively (Figure 2). These numbers represent conversion of 0.63% of DAD and 0.60% of DPD to their respective thiols. Thus, free sulfhydryl involvement in the inhibition of HAA formation is unlikely. It is

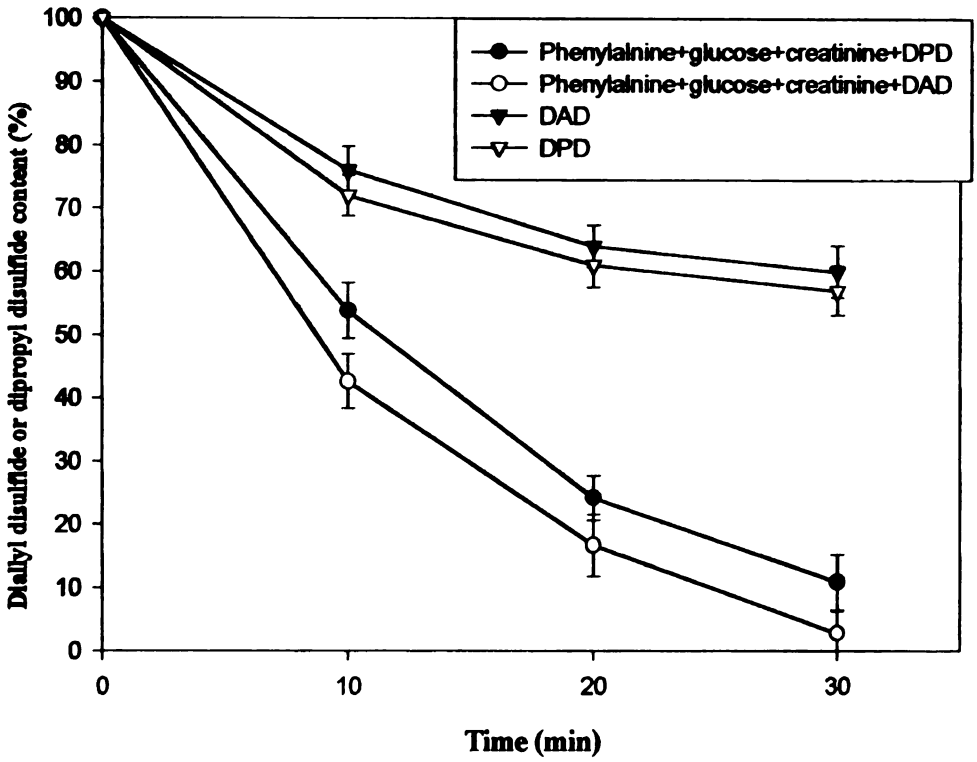


Figure 1. Effect of heating at 180°C for 30 min on the concentrations of organosulfur compounds in a model system containing phenylalanine, creatinine, and glucose. All treatments are made in triplicate.

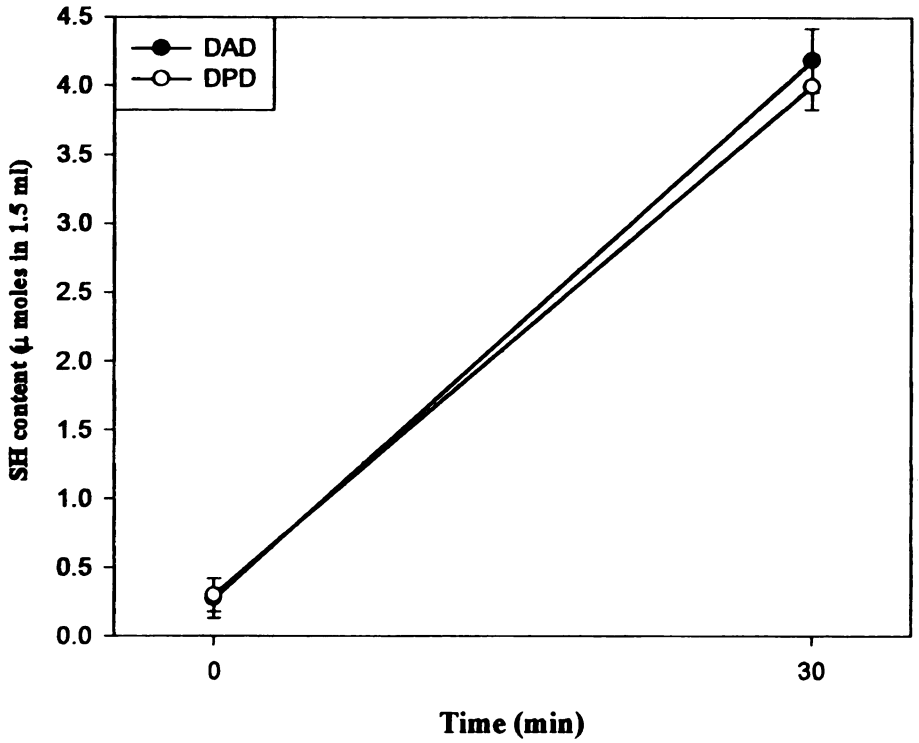


Figure 2. Sulfhydryl content on heating DAD and DPD at 180 °C for 30 min. All treatments were made in triplicate.

more likely that HAA inhibition by the organosulfur compounds occurs by their direct interaction with glucose.

Formation of volatile compounds in heated model systems

In order to determine whether the reaction between DAD and glucose could produce compounds with the potential to inhibit HAA formation, a study was designed to identify some of the principal volatile compounds produced on heating glucose, DAD, and glucose and DAD together, at 180°C for 30 min. Volatile compounds tentatively identified by mass spectrometry included methyl 2-furoate, 5-(hydroxymethyl) 2-furancarboxaldehyde, 2-furancarboxaldehyde, and 1,3-dihydroxy 2-propanone (Table 3, Appendix I). These results generally agree with those of Tai and Ho (1998) and Yu et al. (1994) who reported that 2-furancarboxaldehyde was the major thermal degradation product of glucose. The furfural group is mainly derived via sugar caramelization. Volatile compounds identified from the thermal degradation of DAD are shown in Table 4 and include diallylsulfide, 3-vinyl-1,2-dithiocyclohex-5-ene, diallyltrisulfide, 3-(2,3-dithia-5-hexenyl)-3,4-dihydro-2*H*-thiopyran, 6-methyl-4,5,8,9-tetrathiadodeca-1,11-diene, and 3-(2,3,4,trithia-6-heptenyl)-3,4-dihydro-2*H*-thiopyran. Block et al. (1988) identified several sulfur-containing compounds on heating DAD at 80°C for 2-10 days including thioacrolein dimer 3-vinyl-4*H*-[1,2]-dithin, 2-vinyl-4-*H*-[1,3]-dithin, diallyl sulfide, diallyl tetrasulfide, 6-methyl-4,5,8,9-tetrathiadodeca-1,11-diene, a mixture of 2- and 3-(2',3'-dithia-5'-hexenyl)-3,4-dihydro-2*H*-thiopyran, and 4,5,9,10-tetrathiatrideca-1,12-diene.

Table 3. Compounds tentatively identified on heating glucose at 180°C for 30 min

Peak	Compounds[1]	RT (min)[2]	MW[3]
1	5-(hydroxymethyl) 2-furancarboxaldehyde	16.7	126
2	2-furancarboxaldehyde	23.3	96
3	1,3-dihydroxy 2-propanone	31.1	90
4	Methyl 2-furoate	53.4	126

[1]Identification of the volatile compounds was based on GC/MS analysis.
[2]Retention time.
[3]Molecular weight.

Table 4. Compounds tentatively identified on heating diallyldisulfide at 180°C for 30 min

Peak	Compounds[1]	RT (min)[2]	MW[3]
1	Diallyldisulfide	16.8	146
2	Diallylsulfide	28.1	114
3	3-vinyl-1,2-dithiocyclohex-5-ene	39.3	144
4	Diallyltrisulfide	44.3	178
5	3-(2,3-dithia-5-hexenyl)-3,4-dihydro-2*H*-thiopyran	48.7	218
6	6-methyl-4,5,8,9-tetrathiadodeca-1,11-diene	55.6	252
7	3-(2,3,4-trithia-6-heptenyl)-3,4-dihydro-2*H*-thiopyran	58.7	250

[1]Identification of the volatile compounds was based on GC/MS analysis.
[2]Retention time.
[3]Molecular weight.

Yu et al. (1994) also identified a number of volatile compounds from the thermal degradation of alliin which is the predominant amino acid derivative of garlic and parent compound of DAD. Allyl alcohol was the predominant volatile compound, while others included acetaldehyde, allyl alcohol, acetic acid, thiazole, 2-methylthiazole, dipropyl sulfide, 3-methylthiacyclopentane, 2-formylthiophene, 3-formylthiophene, 2-methyl-1,3-dithiane, 3,6-dimethyl-1,4-dithiane, 4-methyl-1,2-dithiepane, 1,2,3-trithiacyclohexane, 1,2,3,4-tetrathiepane, 4,6-dimethyl-1,2,5-trithiepane, and 4-ethyl-6-methyl-1,2,3,5-tetrathiane.

Compounds identified from the heating of glucose and DAD together at 180°C for 30 min are listed in Table 5. The major compounds were tetrahydrothiophene (THP), 5-methyl-2-thiophene carboxaldehyde, and tetrahydrothiophene-3-one (THT). Thiophene and thiophene-3-one formation from the reaction of glucose and DAD can be explained by the exchange of S and O in the furan ring during heating (Shibamoto et al., 1981). As indicated previously, furan ring products such as 2-furancarboxaldehyde, methyl 2-furoate, and 5-(hydroxymethyl) 2-furancarboxaldehyde are thermal degradation products of glucose. A comparison of the respective GC results (summarized in Table 5 and Appendix I) reveals the formation of compounds assessing from the interaction of glucose and DAD. Because glucose is viewed as a major contributor to HAA formation, it is possible that the reaction of DAD with glucose reduces the availability of glucose to participate in the Maillard reaction, i.e., the carbonylamino reaction. To further verify this hypothesis, the effect of DAD and DPD on HAA formation in a model system containing phenylalanine, creatinine, and glucose and heated at 180°C for 10, 20 or 30 min, was investigated.

Table 5. **Compounds tentatively identified on heating glucose and diallyldisulfide** at 180°C for 30 min

Compounds[1]	RT (min)[2]	MW[3]
Compounds generated on heating glucose		
5-(hydroxymethyl) 2-furancarboxaldehyde	16.7	126
2-furancarboxaldehyde	23.3	96
1,3-dihydroxy 2-propanone	31.1	90
Methyl 2-furoate	53.4	126
Compounds generated on heating diallyldisulfide		
Diallyldisulfide	16.8	146
Diallylsulfide	28.1	114
3-vinyl-1,2-dithiocyclohex-5-ene	39.3	144
Diallyltrisulfide	44.3	178
3-(2,3-dithia-5-hexenyl)-3,4-dihydro-2*H*-thiopyran	48.7	218
6-methyl-4,5,8,9-tetrathiadodeca-1,11-diene	55.6	252
3-(2,3,4-trithia-6-heptenyl)-3,4-dihydro-2*H*-thiopyran	58.7	250
Peak	**Compounds generated on heating glucose and diallyldisulfide**	
1	Tetrahydrothiophene (THP)	26.5	88
2	3,5-diethyl-1,2,4-trithiolane	44.7	180
3	3-(allylthio)-propionic acid	50.4	146
4	Tetrahydrothiophene-3-one (THT)	52.3	102
5	5-methyl-2-thiophene carboxaldehyde	81.4	126
6	9-thianoradamantane	83.1	140

[1]Identification of the volatile compounds was based on GC/MS analysis.
[2]Retention time.
[3]Molecular weight.

Effect of heating time on HAA formation and inhibition of HAA formation in model systems containing DAD and DPD

In order to gain further insight into the inhibition of HAA formation by organosulfur compounds, a study was designed to determine the effect of heating time on HAA formation and inhibition by DAD and DPD in model systems containing phenylalanine, creatinine, and glucose (Figure 3). When HAA formation was evaluated over the entire heating period (30 min), approximately 63% of the HAAs were produced in the initial 10 min of heating. This observation could be explained by the very fast and efficient heat transfer through the wall of the test tube and by the relatively low activation energies (68.9-134.4 kJ/mol) of HAA formation. These results generally agree with those of Arvidson et al. (1997) and, Trompeta and O'Brien (1998) who demonstrated a rapid depletion of glucose in the early stages of the reaction involving phenylalanine, glucose and creatinine. These investigators concluded that glucose was a limiting precursor and actively participated in the formation of HAAs. The retention of creatinine and amino acids was >20%, even after 15 min of heating, while all glucose had reacted after 2.5 min. Chen and Meng (1999) also observed rapid formation of HAAs within the first 5 to 10 min of heating a model system containing glucose, phenylalanine, and creatinine at 150°C and 200°C. After this time, only a steady increase in HAA formation was observed. They concluded that this occurred possibly through the rapid exhaustion of all the HAA precursors in the reaction system. When DAD was added to the system, HAA formation over the first 10 min was significantly ($p<0.05$) decreased (43% reduction). When the model system was heated for 20 min,

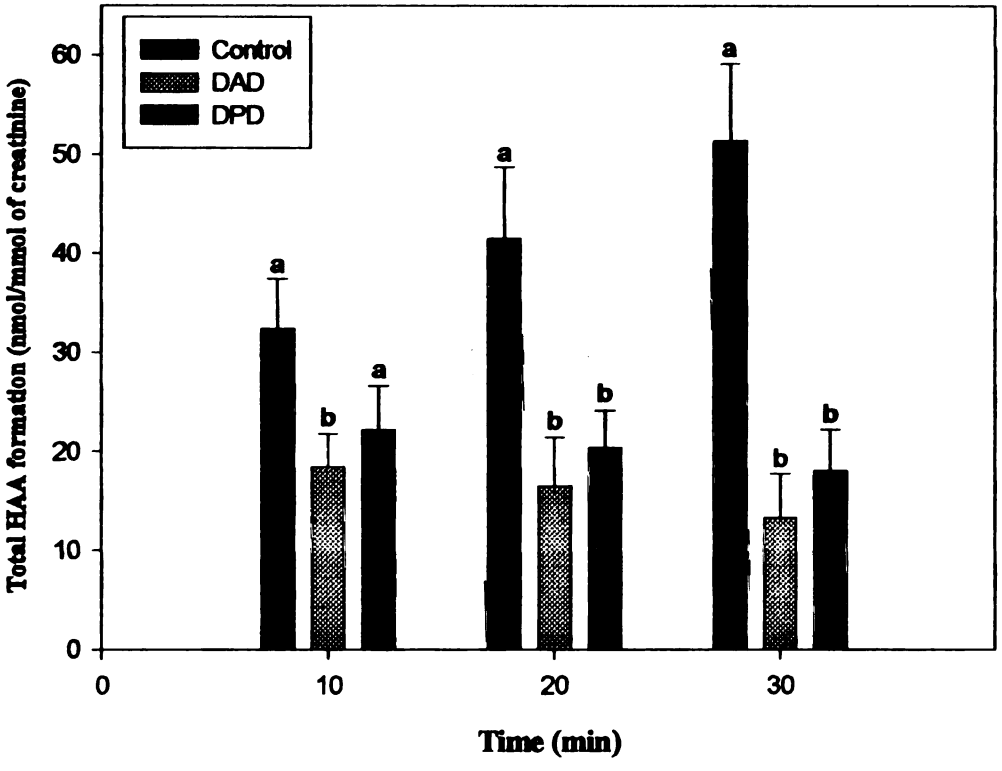


Figure 3. Inhibition of total HAA formation by DAD and DPD in a model system containing phenylalanine, creatinine, and glucose heated at 180°C for 30 min.

Bars with different letters are significantly different ($p<0.05$).
Comparisons are made only within the same column. n=3 for all treatment.

the HAA concentration was approximately 81% of that produced after 30 min of heating. However, the HAA concentrations formed after 10 and 20 min were not significantly different. The addition of DAD and DPD to the model system reduced HAA formation by 60% and 51%, respectively. These results and those of previous studies suggest that HAA inhibition by DAD appears to be through its active interaction with glucose during the first 10 min of heating at 180°C.

Effect of tetrahydrothiophene-3-one (THT) and tetrahydrothiophene (THP) on HAA formation in various model systems

It has been postulated by Tsai et al. (1996) that THT might play an important role in HAA formation. Because of the possible implications of THT or THP as possible inhibitors of HAA formation, the next set of studies investigated their possible role in HAA formation in model systems containing phenylalanine, creatinine and glucose, and glycine, creatinine and glucose.

The principal HAAs formed in the model systems containing glucose, phenylalanine, and creatinine were PhIP, IQx, and MeIQx, whereas PhIP was the only HAA produced in the control model system containing phenylalnine and creatinine (Tables 6 and 7). These results confirm our previous data and also show that THT and THP had no effect on HAA formation in the model systems, regardless of whether glucose was present or not. The major HAAs in the model systems containing glycine, creatinine, and glucose were IQx and MeIQx (Table 8). Scranton (1997) also reported that IQx and MeIQx were the dominant HAAs formed in the same model system under similar heating conditions. The addition of DAD significantly ($p<0.05$) inhibited IQx

Table 6. Effect of tetrahydrothiophene-3-one (THT) and tetrahydrothiophene (THP) on formation of heterocyclic aromatic amines in a model system containing phenylalanine, creatinine, and glucose[1]

	Heterocyclic aromatic amines (nmol/mmol of creatinine)		
Treatment	IQx	MeIQx	PhIP
Control	6.7±2.4[a]	14.6±5.4[a]	30.9±8.7[a]
THT	6.1±2.1[a]	15.7±4.9[a]	31.1±9.1[a]
THP	7.0±2.4[a]	16.0±4.4[a]	33.2±8.5[a]

[a]Means with different superscripts are significantly different ($p<0.05$). Comparisons are made only within the same column. Means±standard deviations; n=3 for all treatments.
[1]Heated at 180°C for 30 min.

Table 7. Effect of tetrahydrothiophene-3-one (THT) and tetrahydrothiophene (THP) on formation of heterocyclic aromatic amines in a model system containing phenylalanine and creatinine[1]

	Heterocyclic aromatic amines (nmol/mmol of creatinine)		
Treatment	IQx	MeIQx	PhIP
Control	ND	ND	11.4±2.7[a]
THT	ND	ND	10.2±2.1[a]
THP	ND	ND	12.8±2.9[a]

[a]Means with different superscripts are significantly different ($p<0.05$). Comparisons are made only within the same column. Means±standard deviations; n=3 for all treatments.
ND= Not detectable. Limit of detection is 0.4 ng.
[1]Heated at 180°C for 30 min.

Table 8. Effect of tetrahydrothiophene-3-one (THT) and tetrahydrothiophene (THP) on formation of heterocyclic aromatic amines in a model system containing glycine, creatinine, and glucose[1]

	Heterocyclic aromatic amines (nmol/mmol of creatinine)	
Treatment	IQx	MeIQx
Control	8.5±1.9[a]	18.3±4.3[a]
DAD	2.3±0.7[b]	5.5±1.7[b]
THT	7.1±2.3[a]	17.4±3.9[a]
THP	8.7±2.7[a]	17.6±4.5[a]

[a]Means with different superscripts are significantly different ($p<0.05$). Comparisons are made only within the same column. Means±standard deviations; n=3 for all treatments.
[1]Heated at 180°C for 30 min.

and MeIQx formation, while THT and THP had no effect on HAA formation. Tsai et al. (1996) postulated that THT might play an important role in IQ-mutagen formation in the reflux boiling of pork juice extracts. They concluded that reductions in the concentrations of THT and the four major Maillard reaction products (pyridines, pyrazines, thiophenes, thiazoles) correlated with a reduction in mutagenicity, even though there was no correlation with mutagenicity when each Maillard reaction product was examined alone. However, our data indicate that THT and THP are merely reaction products between glucose and DAD, and do not influence HAA formation. This divergence of opinion is probably due to the different model systems used and misleading conclusions of Tsai et al. (1996) from insufficient supporting data. They proposed the possible involvement of THT in IQ-mutagen formation in boiled pork juice without studying the specific role of THT on HAA formation. The results of our study confirm that THT and THP are merely reaction products and are not involved in HAA formation.

SUMMARY AND CONCLUSIONS

The results of these studies can be summarized as follows:

1. Selected volatile organosulfur compounds and sodium bisulfite are effective inhibitors of HAA formation in model systems containing phenylalanine, glucose, and creatinine.
2. These compounds are not effective inhibitors of HAA formation in model systems that do not contain glucose.

3. Disulfides react directly with glucose to produce a number of sulfur-containing compounds.
4. Such compounds (i.e., THP and THT) have no influence on the formation of HAAs in the model systems.

While these experiments point to a competitive reaction between organosulfur compounds and amino acids for glucose, the mechanism by which these compounds inhibit HAA formation is still not clarified. However, the observation that DAD has no effect on HAA formation in model systems without glucose provides supporting evidence that the interaction of DAD with glucose is a possible key element in its inhibition of HAA formation. It is also apparent that the products of interaction of glucose and DAD are not directly involved in the inhibition reaction.

CHAPTER FIVE

SUMMARY AND CONCLUSIONS

A series of studies were conducted to investigate the effect of sulfur compounds on HAA formation in meat and model systems, with the overall goal of a fuller understanding of how organosulfur compounds inhibit HAA formation. The reaction between sugar, amino acids, and creatinine in meats leads to the formation of carcinogenic/mutagenic HAAs. These compounds generally are formed from pyrazines and pyridines that are produced by the Maillard reaction. However, as determined in this study, the formation of HAAs in meat and model systems were inhibited by the presence of organosulfur compounds.

The effects of selected organosulfur compounds (DAD, DPD, DAS, AMS, AM, cystine, and cysteine) on HAA formation in fried ground beef were studied. Organosulfur compounds were added directly to 100g ground beef and fried at 225°C for 10 min per side. The HAAs were extracted and purified using solid-phase extraction, and analyzed by HPLC. The identities of the peaks were established by comparing their retention times in UV chromatograms with those of standard references. The inhibitory effects of DAD and DPD were greater than those afforded by cysteine and cystine and the other volatile sulfur compounds that were evaluated. The addition of DAD and DPD to ground beef patties inhibited PhIP formation by 81% and 69%, respectively. DAD and DPD also inhibited DiMeIQx formation in fried ground beef patties by 79% and 62%, respectively. This study clearly demonstrated

that sulfur compounds may represent an alternative approach to reducing HAA formation in cooked beef patties.

The effects of sulfur compounds on HAA formation and overall mutagenicity of fried ground beef patties, as determined by the Ames *S. typhimurium* assay, was studied. Reduction of overall mutagenicity was related to the decrease in HAA formation in fried ground beef patties. The addition of DAD and DPD to ground beef patties inhibited total HAA formation by 78% and 70%, respectively. Again, inhibition of HAA formation by DAD was higher than that of all the other organosulfur compounds (DPD, DAS, AMS, AM, cysteine, and cystine). DAD and DPD significantly ($p < 0.05$) reduced overall mutagenicity by 75% and 65%, respectively, the number of revertants being lowered from 905 revertants/g of meat to 226 and 321 revertants/g of meat, respectively. The addition of DAS, AMS, and AM also significantly ($p < 0.05$) reduced mutagenicity, with reductions of 56%, 43%, and 30%, respectively, while cysteine and cystine did not significantly reduce mutagenicity of the cooked meat patties. The measured mutagenicity in each sample was quite similar to the mutagenicity values calculated from the determined concentrations of HAAs. This study demonstrated that the addition of sulfur compounds did not result in the formation of other mutagenic compounds in the ground beef through the interaction of the potential inhibitor with meat components, or by the thermal breakdown of the inhibitor itself.

A third study was designed to investigate more fully how organosulfur compounds inhibit HAA formation in meat and model system. Addition of glucose to a model system containing phenylalanine and creatinine increased concentrations of PhIP

by 3-4 fold and also increased the formation of other HAAs. These studies established glucose as a major contributor to HAA formation. Volatile organosulfur compounds and sodium bisulfite were effective inhibitors of HAA formation in model systems containing phenylalanine, glucose, and creatinine. However, these compounds did not affect HAA formation in model systems that did not contain glucose. Because the inhibition of the Maillard reaction by sodium bisulfite has been established through its direct interaction with reducing sugars, it can be inferred that a possible mechanism of HAA inhibition by DAD and DPD in model systems containing glucose could be through their interaction with glucose. DAD and DPD concentrations in the model systems containing glucose, creatinine, and phenylalanine decreased by 57 and 46%, even after only 10 min of heating at 180°C. These decreases may be explained by the interaction of the sulfur compounds with components of the model system and/or by their own thermal degradation.

A number of sulfur-containing compounds such as THT and THP were produced on heating glucose and DAD at 180°C for 30min. HAA formation increased rapidly during the first 10 min of heating, followed by a slower increase. HAA inhibition by DAD is possibly through its active interaction with glucose during this same heating period. The products of DAD and glucose interaction, THT and THP, had no effect on HAA formation in the various model systems, regardless of whether glucose was present or not. It was concluded that THT and THP are merely reaction products between glucose and DAD and have no influence on HAA formation.

While these experiments point to a competitive reaction between organosulfur compounds and amino acids for glucose, the mechanism by which these compounds

inhibit HAA formation is still not clarified. However, the observation that DAD does not influence on HAA formation in model systems that do not contain glucose provides supporting evidence that the interaction of DAD with glucose is a possible key element in its inhibition of HAA formation. It is also apparent that the products of interaction of glucose and DAD are not directly involved in the inhibition reaction.

CHAPTER SIX

FUTURE RESEARCH

The effect of organosulfur compounds on HAA formation in meat and model systems and overall mutagenicity in fried beef patties was studied. Results indicate that the addition of sulfur compounds to prior to cooking may represent an alternative approach to reduce HAA formation in cooked beef patties. However, this study revealed other questions that require some further address.

1. The role of organosulfur compounds in inhibiting HAA formation in cooked meat system has been established. However, sensory studies must be carried out to determine an acceptable level of garlic added to ground beef. Similar studies should be done with the individual organosulfur compounds that have demonstrated an ability to reduce HAA formation in cooked meat products.
2. It is well established that DAD and glucose produce several thermal reaction products such as THT and THP. Studies are needed to determine how sulfur compounds interact with glucose. Using ^{35}S-labelled DAD or ^{14}C-labelled glucose to produce thermal reaction products may be an alternative approach to further evaluate/confirm the mechanism of DAD inhibition of HAA formation.
3. This study focused on the principal sulfur compounds present in garlic. Additional investigations should be carried out with other sulfur-containing compounds such as glutathione, glutathionesulfonic acid, cysteine, cysteic acid, and cysteinesulfinic acid. Such studies would enable us to more fully

understand why certain sulfur compounds in food afford greater inhibition of HAA formation than do others.

REFERENCES

Abdulkarim, B.G., and Smith, J.S. 1998. Heterocyclic amines in fresh and processed meat products. J. Agric. Food Chem. 46:4680-4687.

Alink, G., Knize, M., Shen, N., Hesse, S. and Felton, J. 1988. Mutagenicity of food pallets from human diets in the Netherlands. Mutation Res. 206:387-393.

Aoyama, T., Gelboin, H., and Gonzalez, F. 1990. Mutagenic activation of 2-amino-3-methyl-imidazo[4,5-*f*]quinoline by complementary DNA-expressed human liver P450. Cancer Res. 50:2060-2063.

Ames, B.N., McCann, J., and Yamasaki, E. 1975. Methods for detecting carcinogens and mutagens with *Salmonella*/mammalian-microsomal mutagenicity test. Mutation Res. 31:347-364.

Arvidsson, P., Van Boekel, M.A.J.S., Skog, K., and Jägerstad, M. 1997. Kinetics of formation of polar heterocyclic amines in a meat model system. J. Food Sci. 62:911-916.

Arvidsson, P., Van Boekel, M.A.J.S., Skog, K., Solyakov, A., and J☺gerstad, M. 1999. Formation of heterocyclic amines in a meat juice model system. J. Food Sci. 64:216-221.

Augusti, K., and Sheela, C. 1996. Antiperoxide effect of S-alkylcysteine sulfoxide, an insulin secretagogue, in diabetic rats. Experientia. 52:115-120.

Balogh, Z., Gray, J.I., Gomaa, E.A., and Booren, A.M. 2000. Formation and inhibition of heterocyclic aromatic amines in fried ground beef patties. Food Chem. Toxicol. 38:395-401.

Barnes, W.S., Maher, J.C., and Weisburger, J.H. 1983. High-pressure liquid chromatographic method for the analysis of 2-amino-3-methyl[4,5-*f*]imidazo-quinoline, a mutagen formed during the cooking of food. J. Agric. Food Chem. 31:883-886.

Becher, G., Knize, M.G., Nes, F.I., and Felton, J.S. 1988. Isolation and identification of mutagens from a fried Norwegian meat product. Carcinogenesis 9:247-253.

Bjeldanes, L.F., Morris, M.M., Felton, J.S., Stuermer, D., Berry, P., Timourian, H., and Hatch, F.T. 1982. 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., Timourian, H., and Hatch, F.T. 1983. Effects of meat composition and cooking conditions on mutagen formation in fried ground beef. J. Agric. Food Chem. 31:18-21.

Block, E., Lyer, R., Grisoni, S., Saha, C., Belman, S., and Lossing, F. 1988. Lipoxygenase inhibitors from the essential oil of garlic. Markovnikov addition of the allyldithio radical to olefins. J. Am. Chem. Soc. 110:7813-7827.

Block, E. Naganathan, S., Putnam, D., and Zhao, S. 1992. Allium chemistry. J. Agric. Food Chem. 40:2418-2430.

Block, E., Naganathan, S., Putnam, D., and Zhao, S. 1993. Organosulfur chemistry of garlic and onion: recent results. Pure Appl. Chem. 65:625-632.

Block, E., Birringer, M., Jiang, W., Nakahodo, T., Thompson, H., Toscano, P., Uzar, H., Zhang, X., and Zhu, Z. 2001. *Allium* chemistry: Synthesis, natural occurance, biological activity, and chemistry of se-alk(en)ylselenocysteines and their γ-glutamyl derivatives and oxidation products. J. Agric. Food Chem. 49:458-470.

Botting, K.J., Young, M.M., Pearson, A.E., Harris, P.J., and Ferguson, L.R. 1999. Antimutagens in food plants eaten by Polynesians: Micronutrients, phytochemicals and protection against bacterial mutagenicity of the heterocyclic amine 2-amino-3-methylimidazo(4,5-*f*)quinoline. Food Chem. Toxicol. 37:95-103.

Bordia, A., Verma, S., and Srivastava, K. 1998. Effect of garlic (Allium sativum) on blood lipids, blood sugar, fibringen and fibrinolytic activity in patients with coronary artery disease. Prostaglandins Leukotrienes and Essential Fatty Acids. 58:257-263.

Britt, C., Gomaa, E.A., Gray, J.I., Booren, A.M. 1998. Influence of cherry tissue on lipid oxidation and heterocyclic aromatic amine formation in ground beef patties. J. Agric. Food Chem. 46:4891-4897.

Butler, M., Iwasaki, M., Guengerich, F., and Kadlubar, F. 1989. Human cytochrome P-450 (P-450IA2), the phenacetin O-deethylase, is primarily responsible for the hepatic 3-dimethylation of caffeine and N-oxidation of carcinogecic arylamines. Proceedings of the National Academy of Science of the USA. 86:7696-7700.

Cavallito, C., and Bailey, J. 1944. Allicin, the antibacterial principle of *Allium sativium*. I. Isolation, physical properties, and antibacterial action. J. Am. Chem. Soc. 66:1950-1951.

Cavallito, C., Bailey, J., and Buck, J. 1945. The antibacterial principle of *Allium sativium*. 1944. III. Its precursor and "essential oil of garlic" J. Am. Chem. Soc. 67:1032-1033.

Cavallito, C., Buck, J., and Suter, C. 1944. Allicin, the antibacterial principle of *Allium sativium*. II. Determination of the chemical structure. J. Am. Chem. Soc. 66:1952-1954.

Chen, C. 1988. Factors influencing mutagen formation during frying of ground beef. Ph.D. Dissertation, Michigan State University, East Lansing, MI, USA

Chen, Y., Zheng, R., Jia, Z., and Ju, Y. 1990. Flavonoids as superoxide scavengers and antioxidants. Free Radical Biol. Med. 9:19-21.

Chen, C., Pearson, A.M., and Gray, J.I. 1992. Effect of different antioxidants on formation of meat mutagens during frying of ground beef. Proc. 38th Intern. Congr. Meat Sci. Technol. 3:567-570, August 23-28, Clermont-Ferramd. France.

Chen, B.H., and Meng, C.N. 1999. Formation of heterocyclic amines in a model system during heating. J. Food Protect. 62:1445-1450.

Dashwood, R.H., Xu, M., Hernaez, J.F., Hasaniya, N., Youn, K., Razzuk, A. 1999. Cancer chemopreventive mechanism of tea against heterocyclic amine mutagens from cooked meat. Proc. Soc. Exp. Biol. Med. 220:239-243.

De Flora, S., Benicelli, C., Serra, D., Izzotti, A., Cesarone, C. Role of glutathione and N-acetylcysteine as inhibitors of mutagenesis and carcinogenesis. In Absorption and utilization of amino acids., Friedman, M., Ed. CRC, Boca Raton, FL, 1989. 3:19-53.

Deshpande, R., Khan, M., Bhat, D., and Navalkar, R. 1993. Inhibition of *Mycobacterium avium* complex isolates from AIDS patients by garlic (Allium sativum). J. Antimicrob. Chemother. 32:623-626.

Dragsted, L.O. 1992. Exposure and carcinogenicity of heterocyclic amines. Proceedings of the Toxicology Forum, 1992 Annual Meeting, Copenhagen, Denmark, 141-148.

Edmonds, C.G., Sethi, S.K., Yamaizumi, Z., Kasai, H., Nishimura, S., and McCloskey, J.A. 1986. Analysis of mutagen from cooked foods by directly combined liquid chromatography-mass spectrometry. Environ. Health Perspect. 67:35-40.

Esumi, H., Ohgaki, H., Kohzen, E., Takayama, S., and Sugimura, T. 1989. Inducfion of lyinphoma in CDFI mice by the food mutagen, 2-amino-lmethyl-6-phenyl-imidazo[4,5-*b*]pyridine. Jpn. J. Cancer Res. 80:1176-1178.

Faulkner, J.A. 1994. Mechanism of heterocyclic amine formation in fried ground beef-the role of oxidized lipid and the Maillard reaction. Ph.D. Dissertation. Michigan State University, East Lansing, MI USA.

Felton, J.S., Knize, M.G., Wood, C., Wuebbles, B.J., Healy, S.K., Stuermer, D., 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., Anderson, B.D., Bjeldanes, L.F., and Hatch, F.T. 1986a. Identification of the mutagens in cooked beef. Environ, Health Perspect. 67:17-24.

Felton, J.S., Knize, M.G., Shen, N.H., Lewis, P.R., Andresen, B.D., Happe, J., and Hatch, F.T. 1986b. The isolation and identification of a new mutagen from firied ground beef. 2-amino-l-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP). Carcinogenesis 7:1081-1086.

Felton, J.S., and Knize, M.G. 1990. Heterocyclic amine mutagens/carcinogens in foods. In Handbook of Experimental Pharmacology. Eds., C.S. Cooper and P.L. Grover. pp.471-502. Springer-Verlag, Berlin.

Felton, J.S., and Knize, M.G. 1991. Occurrence, identification, and bacterial mutagenicity of heterocyclic amines in cooked food. Mutation Res. 259:205-217.

Felton, J.S., Knize, M.G., Roper, M., Fultz, E., Shen, N.H., and Turteltaub, K.W. 1992. Chemical analysis, prevention and low-level dosimetry of heterocyclic amines from cooked food. Cancer Res. (Suppl.) 52:2103s-2107s.

Felton, J.S., Fultz, E., Dolbeare, F.A., and Knize, M.G. 1994. Effect of microwave pretreatment on heterocyclic aromatic amine mutagens/carcinogens in fried beef patties. Food Chem. Toxicol. 32:897-903.

Felton, J.S., Malfatti, M., Knize, M.G. Salmon, C.P., Hopmans, E., and Wu, R. 1997. Health risks of heterocyclic amines. Mutation Res. 376:37-41.

Folch, J., Lees, M., and Stanley, G. H. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497-509.

Friedman, M., Wehr, C., Schade, J., MacGregor, J. 1982. Inactivation of aflatoxin B_1 mutagenecity by thiols. Food Chem. Toxicol. 20:887-892.

Friedman, M., and Molnar-Perl, I. 1990. Inhibition of browning by sulfur amino acids. 1. heated amino acid-glucose system. J. Agric. Food Chem. 38:1642-1647.

Friedman, M. 1996. Food browning and its prevention: An overview. J. Agric. Food Chem. 44:631-653.

Ghannonoum, M. 1988. Studies on the anticandidal mode of action of *Allium sativum* (garlic). J. Gen. Microbiol. 134:2917-2924.

Grivas, S., Nyhammar, T., Olsson, K., and J☺gerstad, M. 1985. Formation of a new mutagenic DiMeIQx compound in a model system by heating creatinine, alanine and fructose. Mutation Res. 151:177-183.

Grivas, S., Nyhammar, T., Olsson, K., and J☺gerstad, M. 1986. Isolation and identification of the food mutagens IQ and MeIQx from a heated model system of creatinine, glycine, and glucose. Food Chem. 20:127-136.

Gross, G.A., Philippossian, G., and Aeschecher, 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.

Gross, G.A., and Grüter, A. 1992. Quantitation of mutagenic/carcinogenic heterocyclic amines in food products. J. Chromatogr. 592:271-278.

Gross, G.A, Turesky, R.J., Fay, B.L., Stillwell, W.G., Skipper, P.L., and Tannenbaum, S.R. 1993. Heterocyclic aromatic amine formation in grilled bacon, beef and fish and in grill scrapings. Carcinogenesis 14:2313-2318.

Gry, J., Vahl, M., and Nielsen, P. 1986. Mutagens in Fried Meat Publ. No. 139, National Food Administration, Copenhagen, Denmark.

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, E.T., Felton, J.S., and Bjeldanes, L.F. 1982. Mutagens from the cooking of food: Thermic mutagens in beef. In Carcinogens and Mutagens in the Environment. Vol.1. Ed., Stich, H.F. pp. 147-173. Critical Reviews of Toxicology. CRC Press, Boca Raton, FL.

Hayatsu, H., Arimotao, S., and Wakabayashi, K. 1991. Methods for separation and detection of heterocyclic amine. In Mutagens in Food & Detection and Prevention. Eds., H. Hayatsu. pp. 101-112. CRC Press, Boca Raton, FL.

Hirose, M., Takahashi, S., Ogawa, K., Futakuchi, M., and Shirai, T. 1999. Phenolics: Blocking agents for heterocyclic amine-induced carcinogens. Food Chem. Toxicol. 37:985-992.

Ho, C.T., Chen, Q., Shi, H., Zhang, K.Q., and Rosen, R.T. 1992. Antioxidative effect of polyphenol extract prepared from various Chinese teas. Prev. Med. 21:520-525.

Hodge, J.E. 1953. Chemistry of browning reactions in model systems. J Agric. Food Chem. 1:928-943.

Holme, J., Walline, H., Brunborg, G., Soderlund, E., Hongslo, J., and Alexander, J. 1989. Genotoxicity of the food mutagen 2-amino-1-methyl-6-phenylimidazo [4,5-*b*] pyridine (PhIP): formation of 2-hydroxyamino-PhIP, a directly acting genotoxic metabolite. Carcinogenesis. 10:1389-1395.

Hughes, B., and Lawson, L. 1991. Antimicrobial effects of *Allium sativum L.* (garlic), *Allium ampeloprasum* (elephant garlic), and *Allium cepa L.* (onion), garlic compounds and commercial garlic supplement products. Phytother. Res. 5:154-158.

Iberl, B., Winkler, G., and Knobloch, K. 1990. Products of allicin transformation: ajoenes and dithiins, characterization and their determination by HPLC. Planta. Med. 56:202-211.

Jägerstad, M., Reutersward, L.A., Olsson, R., Grivas, S., Nyhammer, T., Olsson, K., Oste, R., and Dahlqvist, A. 1983a. Creatin(in)e and Maillard reaction products as precursors of mutagenic compounds: effects of various amino acids. Food Chem. 12:255-264.

Jägerstad, M., Reutersward, L.A., Oste, R., Dahlqvist, A., Grivas, S., Olsson, K., and Nyhammar, T. 1983b. Creatinine and Maillard reaction products as precursors of mutagenic compounds formed in fried beef. In The Maillard Reaction in Food and Nutrition. ACS Symposium Series 215. Eds., Walleer, G., and Feather, M. pp.507-519. American Chemical Society, Washington, DC.

Jackson, S., Hargraves, A., Stroup, W., and Diachenko, G. 1994. Heterocyclic aromatic amine content of selected beef flavors. Mutation Res. 320:113-124.

Johansson, M.A.E., and Jägerstad, M. 1993. Influence of oxidized deep-frying fat and iron on the formation of food mutagens in a model system. Food Chem. Toxicol. 31:971-979.

Johansson, M.A.E., and Jägerstad, M. 1994. Occurrence of mutagenic/carcinogenic heterocyclic amines in meat and fish products, including pan residues, prepared under domestic conditions. Carcinogenesis 15:1511-1518.

Jones, R.C., and Weisburger, J.H. 1988. Inhibition of aminoimidazoquinoxaline type and aminoimidazol-4-one-type mutagen formation in liquid reflux models by L-tryptophan and other selected indoles. Jpn. J. Cancer Res. 79:222-230.

Kabelik, J. 1970. Antimikrobielle eigenschaften des knoblauchs. Pharmazie. 25:266-270.

Kamanna, V., and Chandrasekhara, N. 1984. Hypocholesteremic activity of different fractions of garlic. Indian J. Med. Res. 79:580-583.

Kasai, H., Yatnaizumi, Z., Wakabayashi, K., Nagao, M., Sugitnura, T., Yokoyatna, S., Miyazawa, T., Spingam, N., Weisburger, J., and Nishimura, S. 1980. Potent novel mutagens produced by broiling fish under normal conditions. Proceedings of the Japan Academy 56: 278-283.

Kasai, H., Yamaizumi, Z., Nishimura, S., Wakabayashi, K., Nagao, M., Sugimura, T., spingarn, N., Weisburger, J., Yokoyama, S., and Miyazawa, T. 1981a. A potent mutagen in broiled fish. Part1. 2-amino-3methyl-3H-imidazo(4,5-*f*)quinoline. J. Chem. Soc. 2290-2293.

Kasai, H., Ywnaizumi, Z., Shiomi, T., Yokoyama, S., Miyazawa, T., Wakabayashi, K., Nagao, M., Sugimua, T., and Nishimura, S. 1981b. Structure of a potent mutagen isolated from fried beef. Chem. Lett. 4:485-488.

Kato, T., Kikugawa, K., and Hayatsu, H. 1986. Occurrence of the mutagens 2-amino-3,4,8-trimethyl-imidazo(4,5-*f*)quinoxaline (4,8-MeIQx) in Japanease smoked, dried fish products. J. Agric. Food Chem. 34:810-814.

Kato, T., Michikoshi, K., Minowa, Y., Maeda, Y., and Kikugawa, K. 1998. Mutagenicity of cooked hamburger is reduced by addition of onion to ground beef. Mutation Res. 420:109-114.

Kendler, B. 1987. Garlic (*Allium sativium*) and onion (*Allium cepa*): A review of their relationship to cardiovascular disease. Prev. Med. 16:670-685.

Kikugawa, K., Kato, T., and Hayatsu, H. 1986. The presence of 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline in smoked dry bonito (katsuobshi). Jpn. J. Cancer Res. 7:99-102.

Kim, I.S., Wakabayashi, K., Kurosaka, R., Yamaizumi, Z., Jinno, F., Koyota, S., Tada, A., Nukaya, H., Takahashi, M., Sugimura, T., and Nagao, M. 1994. Isolation and identification of a new mutagen, 2-amino-4-hydroxy-methyl-3,8-dimethylimidazo (4,5-*f*)quinoxaline (4-CH_2OH-8-MeIQx), from beef extract. Cacinogenesis. 15:21-26.

Kitada, M., Taneda, M., Ohta, K., Nagashima, K., Itahashi, K., and Kamataki, T. 1990. Metabolic activation of aflatoxin B_1 and 2-amino-3-methyl-imidazo[4,5-*f*]quinoline by human adult and fetal livers. Cancer Res. 50:2641-2645.

Kitada, M., Taneda, M., Itahashi, K., and Kamataki, T. 1991. Forms of cytochrome P450 in human fetal liver: purification and their and their capacity to activate promutagens. Jpn. J. Cancer Res. 82:426-432.

Knize, M.G., Roper, M., Shen, H.N., and Felton, J.S. 1990. Proposed structure for an amino-dimethylimidazofuropyridine mutagen in cooked meats. Carcinogenesis. 11:2259-2262.

Knize, M.G., Hopmans, E., and Happe, J.A. 1991. The identification of a new heterocyclic amine mutagen from a heated mixture of creatine, glutamic acid and glucose. Mutation Res. 260:313-319.

Knize, M.G., Dolbeare, F.A., Carroll, K.L., Moore, D.H., and Felton, J.S. 1994. Effect of cooking time and temperature on the heterocyclic amine content of fried-beef patties. Food Chem. Toxicol. 32:595-603.

Knize, M.G., Salmon, C.P., Hopmans, E.C., and Felton, J.S. 1997. Analysis of foods for heterocyclic aromatic amine carcinogens by solid-phase extraction and high performance liquid chromatography. J. Chromatogr. 763:179-185.

Knize, M.G., Sinha, R., Brown, E.D., Salmon, C.P., Levander, O.A., Felton, J.S., and Rothman, N. 1998. Hetrocyclic amine content in restaurant-cooked hamburgers, steaks, ribs, and chicken. J. Agric. Food Chem. 46:4648-4651.

Knize, M.G., Salmon, C.P., Pais, P., and Felton, J.S. 1999. Food heating and the formation of heterocyclic aromatic amine and polycyclic aromatic hydrocarbon mutagens/carcinogens. Adv. Exp. Med. Biol. 459:179-193.

Knowles, L., and Milner, J. 1998. Depressed p34cdc kinase activity and G2/M phase arrest induced by diallyl disulfide in HCT-15 cells. Nutrition and Cancer. 30:169-174.

Kritchevsky. D. 1991. The effect of dietary garlic on the development of cardiovascular disease. Trends Food Sci. Technol. 2:141-144.

Krone, C., and Iwaoka, W., 1987. Commercial food processing operation and mutagen formation. J. Food Protect. 50:167-173.

Kurosaka, R., Wakabayashi, K., Ushiyama, H., Nukaya, H., Arakawa, N., Sugimura, T., and Nagao, M. 1992. Detection of 2-amino-1-methyl-6-(4-hydroxyphenyl)-imidazo[4,5-*b*]pyridine in broiled beef. Jap. J. Cancer Res. 83:919-922.

Lansen, J., Dragsted, L., Frandsen, H., Kristiansen, E., Rasmussen, E. Nielsen, P., and Knudsen, I. 1990. Carcinogenicity of mutagen from cooked meats. In Mutagen and Carcinogens in the Diet. Eds., Pariza, M.W., Aeschbacher, J., Felton, J.S., and Sato, S. pp. 89-108. Wiley-Liss, New York, NY.

Lawson, L., and Hughes, B. 1990. Trans-1-propenyl thiosulfinates: new compounds in garlic homogenates. Planta. Med. 56:589-596.

Lawson, L., Wood, S., and Hughes, B. 1991. HPLC analysis of allicin andd other thiosulfinates in garlic clove homogenates. Planta. Med. 57:263-270.

Lawson, L., Ransom, D., and Hughes, B. 1992. Inhibition of whole blood platelet aggregation by compounds in garlic clove extracts and commercial garlic products. Thromb. Res. 65:141-156.

Lawson, L. 1993. Bioactive organosulfur compounds of garlic and garlic products; role in reducting blood lipids. In Human Medicinal Agents from Plants., ACS Symp. Ser. 534. Eds., A.D. Kinghorn and M.F. Balandrin, pp. 306-330. Am. Chem. Soc. Books, Washington, D.C.

Lawson, L. 1996. The composition and chemistry of garlic cloves and processed garlic. Ch. 3 In Garlic; The Science and Therapeutic Application of *Allium sativum L.* and Related Species. Eds., H.P. Koch and L.D. Lawson, pp. 37-69. Williams & Wilkins, Baltimore, Maryland.

Lee, H., and Tsai, S.J. 1991. Detection of IQ-type mutagens in canned roasted eel. Food Chem. Toxicol. 29:517-529.

Lucier, G., and Lin, B. 2000. Garlic: Flavor of the ages. Agric. Outlook. June-July, 7-10.

Lutomski, J. 1983. Das wichtigste uber knoblauch und knoblauch-praparate. Dtsch. Apoth. Ztg. 123:623-624

Lynch, A.M, Knize, M.G., Boobis, A.R., Gooderham, N.J., Davies, D.S., and Murray, S. 1992. Interindividual variability in systemic exposure in humans to 2-amino-3,8-dimethylimidazo(4,5-*f*)quinoxaline and 2-amino-1-methyl-6-phenylimidazo (4,5-*b*)pyridine, carcinogens present in cooked beef. Cancer Res. 52:6216-6223.

Manabe, S., Kurihara, N., Wada, O., Tohyama, K., and Aramaki, T. 1992. Formation of PhIP in a mixture of creatine, phenylalanine and sugar or aldehyde by aqueous heating. Carcinogenesis 13:827-830.

Milic, B.L., Djilas, S.M., and Canadanovic-Brunet, J.M. 1993. Synthesis of some heterocyclic amino-imidazoazarenes. Food Chem. 46:273-276.

Milner, J. 1996. Garlic: its anticarcinogenic and antimutagenic properties. Nutr. Rev. 54:S82-S86.

Mohammad, S. and Woodward, S. 1986. Characterization of a potent inhibitor of platelet aggregation and release reaction isolated from *Allium sativum* (garlic). Thromb. Res. 44:793-806.

Moore, D., and Felton, J.S. 1983. A microcomputer program for analyzing Ames test data. Mutation Res. 119:95-102.

Mottram, D.S., and Whitfield, F.B. 1995. Maillard-lipid interactions in nonaqueous systems: Volatiles from the reaction of cysteine and ribose with phosphatidylcholine. J. Agric. Food Chem. 43:1302-1306.

Muramatsu, M., and Matsushima, T. 1985. Formation of MeIQx and 4,8-DiMeIQx by heating mixtures of creatinine, amino acids, and monosaccharides. Mutation. Res. (Abstract) 147:266-267.

Murkovic, M., Steinberger, D., and Pfannhauser, W. 1998. Antioxidant spices reduce the formation of heterocyclic amines in fried meat. Z. Lebensm. Unters. Forsch. A. 207:477-480.

Murray, S., Gooderham, N.J., Boobis, A.R., and Davies, D.S. 1987. Trp-P-2 is not detectable in cooked meat and fish. Carcinogenesis. 8:937-940.

Murray, S., Gooderham, N.J., Boobis, A.R., and Davies, D.S. 1988. Measurement of Melox and DiMeIQx in fried beef by capillary column gas chromatography electron capture negative ion chemical ionization mass spectrometry. Carcinogenesis 9:321-325.

Murray, S., Gooderham, N.J., Boobis, A.R., and Davies, D.S. 1993. Quantification of the carcinogens 2-amino-3,8-dimethyl-and 3,4,8-trimethylimidazo(4,5-*f*)quinoxaline and 2-amino-1-methylimidazo(4,5-*b*)pyridine in food using a combined assay based on capillary column gas chromatography-negative ion mass spectrometry. J. Chromatogr. 616:222-219.

Namiki, M., and Hayashi, T. 1975. Development of novel free radicals during the amino-carbonyl reaction of sugars with amino acids. J. Agric. Food Chem. 23(3):487-493.

Namiki, M., and Hayashi, T. 1980. Formation of two-carbon sugar fragment at an early stage of the browning reaction of sugar with amine. Agric. Biol. Chem. 44(11):2575-2579.

Namiki, M., and Hayashi, T. 1981. Formation of novel free radical products in an early stage of Maillard reaction. Progr. Food Nutr. Sci. 5:81-91.

Namiki, M., and Hayashi, T. 1983. A new mechanism of the Maillard reaction involving sugar fragmentation and free radical formation. In The Maillard

Reaction in Foods and Nutrition. Eds., G.R. Waller and M.S. Feather. pp.21-46. American Chemical Society, Washington, DC.

Negishi, C., Wakabayashi, K., Tsuda, M., Sato, S., Sugimura, T., Saito, H., Maeda, M., and Jägerstad, M. 1984. Formation of 2-amino-3,7,8-trimethyl[4,5-*f*]quinoxaline, a new mutagen, by heating mixture of creatine, glucose, and glycine. Mutation Res. 140:55-59.

Neter, J., Wasserman, W., and Whitmore, G.A. 1993. Analysis of variance. In Applied Statistics. pp 651-683. Simon and Schuster, Inc., Needham Heights, MA.

Nielson, K., Mahoney, A., Williams, L., and Rogers, V. 1991. X-Ray fluorescence measurements of Mg, P, S, Cl, K, Ca, Mn, Fe, Cu, and Zn in fruits, vegetables and grain products. J. Food Comp. Analysis. 4:39-51.

Nishimura, H., Wijava, C. H., and Mizutani, J. 1988. Volatile flavor components and antithrombotic agents: Vinyldithiins from *Allium victorialis*. J. Agric. Food Chem. 36:563-566.

Nukaya, H., Koyota, S., Jinno, F., Ishida, H., Wakabayashi, K., Kurosaka, R., Kim, I., Yamaizumi, Z., Ushiyama, H., Sugimura, T., Nagao, M., and Tsuji. 1994. Structural determination of a new mutagenic heterocyclic amine, 2-amino-1,7,9-trimethylimidazo[4,5-*g*]quinoxaline (7,9-DiMeIgQx), present in beef extract. Cacinogenesis. 15:1151-1154.

Nyhammar, T. 1986. Studies on the Maillard reaction and its role in the formafion of food mutagens. Doctoral Thesis, Swedish University of Agricultural Sciences, Uppsala, Sweden. ISBN 91-576-2658-8.

Oguri, A., Suda, M., Totsuka, Y., Sugimura, T., and Wakabayashi, K. 1998. Inhibitory effects of antioxidants on formation of heterocyclic amines. Mutation Res. 402:237-245.

Ohgaki, H., Kusam, K., Matsukura, N., Morino, K., Hasegawa, H., Sato, S., Sugimura, T., and Takayama, S., 1984. Carcinogenicity in mice of a mutagenic compound, 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline, from broiled sardine, cooked beef and beef extract. Carcinogenesis. 5:921-924.

Ohgaki, 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 feeding 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline. Carcinogenesis. 5:921-924.

Ohgaki, H., Hasegawa, H., Suenaga, M., Sato, S., Takayama, S., and Sugimura, T. 1987. Carcinogenicity in mice of a mutagenic compound (MeIQx) from cooked foods. Carcinogenesis. 8:665-668.

Ohgaki, H., Takayama, S., and Sugimua, T. 1991. Carcinogenicities of heterocyclic amines in cooked food. Mutation Res. 259:399-410.

Okamoto, T., Shudo, K., Hashimoto, Y., Kosuge, T., Sugimura, T., and Nishimura, S. 1981. identification of a reactive metabolite of the mutagen, 2-amino-3-methylimidazo(4,5-*f*)-quinoline. Chem. Pharm. Bull. 29:590-593.

Omkumar, R., Kadam, S., Banerji, A., and Ramasarma, T. 1993. On the involvement of intramolecular protein disulfide in the irreversible inactivation of 3-hydroxy-3-methylglutaryl-CoA reductase by diallyl disulfide. Biochimica et Biophysica Acta. 1164:108-112.

Orth-Wagner, S. 1986. Moderne phytotherapie. Dtsch. Apotheker. 38:42-47.

Overvik, E., Kleman, M., Berg, I., and Gustafsson, J. 1989. Influence of creatine, amino acids and water on the forinafion of the mutagenic heterocyclic amines found in cooked meat. Carcinogenesis 10:2293-2301.

Paterson, A.M., and Chipman, J.K. 1987. Activation of 2-amino-3-methylimidazo-(4,5-*f*)quinoline in rat and human hepatocyte/Salmonella mutagenicity assays: the contribution of hepatic conjugation. Mutagenesis. 2:137-140.

Pentz, R., Guo, Z., Kress, G., Műller, B., and Siegers, C.P. 1990. Standardization of garlic powder preparations by the estimation of free and hydrolysable SH groups. Planta. Med. 56:591-598.

Plengvidhay, C., Chinayon, S., Sitprija, S., Pasatrat, S., and Tankeyoon, M. 1988. Effects of spray dried garlic preparation on primary hyperlipoproteinemia. J. Med. Assoc. Thailand. 71:248-252.

Powrie, W., Wu, C., Rosin, M., and Stich, H. 1981. Clastogenic and mutagenic activities of Maillard reaction model systems. J. Food Sci. 46:1433-1438.

Pratt, D.E., and Birac, P.M. 1979. Source of antioxidant activity of soybeans and soy products. J. Food Sci. 44:1720-1722.

Rappaport, S.M., McCartney, M.C., and Wei, E.T. 1979. Volatilization of mutagens from beef during cooking. Cancer Lett. 8:139-145.

Reistad, R., Possland, O., Latva-Kala, K., Rasmussen, T., Vikse, R., Becher, G., and Alexander, J. 1997. Heterocyclic aromatic amines in human urine following a fried meat meal. Food Chem. Toxicol. 35:945-955.

Reuter, H. and Sendl, A. 1994. *Allium sativum* and *Allium ursinum*: chemistry, pharmacology, and medical applications. In Economic and Medicinal Plant Research. pp. 54-113, Academic Press, New York,.

Reutersward, A.L., Skog, K., and Jägerstad, M. 1987. Mutagenicity of pan-fried bovine tissues in relation to their content of creatine, creatinine, monosaccharides and free amino acids. Food Chem. Toxicol. 25:755-762.

Reynolds, T. 1963. Chemistry of nonenzymic browning. I. The reaction between aldoses and amines. Adv. Food Res., 12:1-52.

Rhee, K.S., Donnelly, K.C., and Ziprin, Y.A. 1987. Reduction of mutagen formation in fried ground beef by glandless cottonseed flour. J. Food Protect. 50:753-755.

Rizzi, G. 1994. The Maillard reaction in foods. In Maillard Reactions in Chemistry, Food, and Health. Eds., T.P. Labuza, G.A. Reineccius, V.M. Monnier, J. O'Brein, and J.W. Baynes., pp.11-19. The Royal Society of Chemistry, Thomas Graham House, Science Park, Cambridge.

Saito, K., Shinohara, A., Kamataki, T., and Kato, R. 1985. Metabolic activation of mutagenic N-hydroxyarylamines by O-acetyltranferase in *Samonella typhimurium* TA98. Arch. Biochem. Biophys. 239:286-295.

Salmon, C.P., Knize, M.G., and Felton, J.S. 1997. Effect of marinating on heterocyclic amine carcinogen formation in grilled chicken. Food Chem. Toxicol. 35:433-441.

Schuirmann, E., and Eichner, K. 1991. Formation of aminoimidazoquinolines ans quinoxalines (IQ-compounds) during heating of meat products. In Stategies for Food Quality Control and Analytical Methods in Europe. Eds. W. Baltes, T. Eklund, R. Fenwich, W. Pfannhauseer, A. Ruiter and H.P. Their. pp.739-744. B.Behr's Berlag, Hamburg.

Scranton, L. 1997. Effect of lipids and iron on heterocyclic aromatic amine formation in aqueous model systems. M.S. Thesis. Michigan State University, East Lansing, MI, USA

Semmler, F. 1892. Uber das Atherische Ol des Knoblauchs (*Allium sativium*). Arch. Pharm. 230:434-443.

Shashikanth, K., Basappa, S., and Sreenivasamurthy, V. 1986. Effect of feeding raw and boiled garlic (*Allium sativum L.*) extracts on the growth, caecal microflora, and serum proteins of albino rats Nutr. Rep. Int. 33:313-319.

Sheen, L.Y., Wu, C.C., Lii, C.K., and Tsai, S.J. 1999. Metabolite of diallyl disulfide and diallyl sulfide in primary rat hepatocytes. Food Chem. Toxicol. 37:1139-1146.

Shibamoto, T., Nishimura, O., and Mihara, S. 1981. Mutagenicity of products obtained from a maltol-ammonia browning model system. J. Agric. Food Chem. 29:643-646.

Shioya, M., Wakabayashi, K., Sato, S., Nagao, M., and Sugimura, T. 1987. Formation of a mutagen, 2-amino-l-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) in cooked beef, by heating a mixture containing creatinine, phenylalanine and glucose. Mutation Res. 191:133-138.

Sichel, G., Corsaro, C., Scalia, M., Di Bilo, A.J., and Bonomo, R.P. 1991. In vitro scavenger activity of some flavonoids and melanins against O^{2-}. Free Radical Biol. Med. 11:1-8.

Sinha, R., Knize, M.G., Salmon, C.P., Brown, E.D., Rhodes, D., Felton, J.S., Levander, O.A., and Rothman, N. 1998. Heterocyclic amine content of pork products cooked by different methods and to varying degree of doneness. Food Chem. Toxicol. 36:289-297.

Singh, A., and Shukla, Y. 1998. Antitumor activity of diallyl sulfide on polycyclic aromatic hydrocarbon-induced mouse skin carcinogenesis. Cancer Letters. 131:209-214.

Sitprija, S., Plengvidhya, C., Kangkaya, V., Bhuvapanich, S., and Tunkayoon, M. 1987. Garlic and diabetes mellitus phase II clinical trial. J. Med. Assoc. Thailand. 70:223-227.

Skog, K., and Jägerstad, M. 1990. Effects of monosaccharides and disaccharides on the formation of food mutagens in model systems. Mutation Res. 25:263-272.

Skog, K, and Jägerstad, M. 1991. Effect of glucose on the formation of PhIP in a model system. Carcinogenesis. 12:2297-2300.

Skog, K., Jägerstad, M. and Laser-Reutersward, A. 1992. Inhibitory effect of carbohydrates on the formation of mutagens in fried beef patties. Food Chem. Toxicol. 30:681-688.

Skog, K. 1993. Cooking procedures and food mutagens: A literature review. Food Chem. Toxicol. 31:655-675.

Skog, K., and Jägerstad, M. 1993. Incoporation of carbon atoms from glucose into the food mutagens MeIQx and 4,8-DiMeIQx using ^{14}C-labelled glucose in a model system. Carcinogenesis. 14:2027-2031.

Skog, K., Steineck, G., Augustsson, K., and Jägerstad, M. 1995. Effect of cooking temperature on the formation of heterocyclic amines in fried meat products and pan residues. Carcinogenesis. 16:861-867.

Skog, K., Augustsson, K., Steineck, G., Stenberg, M., and Jägerstad, M. 1997. Polar and nonpolar heterocyclic amines in cooked fish and meat products and their corresponding pan residues. Food Chem. Toxicol. 35:555-565.

Skog, K., Solyakov, A., Arvidsson, P., and Jägerstad, M. 1998. Analysis of nonpolar heterocyclic amines in cooked foods and meats extracts using gas chromatography-mass spectrometry. J. Chromatogr. 803:227-233.

Skog, K., Solyakov, A., and Jägerstad, M. 2000. Effects of heating conditions and additives on the formation of heterocyclic amines with reference to amino-carbolines in a meat juice model system. Food Chem. 68:299-308.

Smith, A.K., and Circle, S.J. 1978. Soybeans. In Chemistry and Technology. Avi Publishing Co., Westport, CT.

Snyderwine, E.G., Roller, P.P., Wirth, P.J., Adamson, R.H., Sato, S., and Thorgeirsson, S.S. 1987. Synthesis, purification and mutagenicity of 2-hydroxyamino-3-methylimidazo[4,5-*f*]quinoline. Carcinogenesis 8:1017-1020.

Sorata, Y., Takahama, U., Kimura, M. 1984. Protective effect of quercetin and rutin on photosensitized lysis of human erythrocytes in the presence of hematoporphyrin. Biochem. Biophys. Acta. 799:313-317.

Sparnins, V.L., Barany, G., and Watternberg, L.W. 1988. Effects of organosulfur compounds from garlic and onions on benzo[*a*]pyrene-induced neoplasia and glutathione-S-transferase activity in the mouse. Carcinogenesis. 9:131-134.

Spingarn, N.E., and Garvie, C.T. 1979. Formation of mutagens in sugar-ammonia model systems. J. Agric. Food Chem. 27:1319-1321.

Stavric, B. 1994. Biological significance of trace levels of mutagenic heterocyclic aromatic amines in human diet: a critical review. Food Chem. Toxicol. 32:977-994.

Stoll, A. and Seebeck, E. 1947. Űber Allin, die genuine Muttersubstanz des Knoblauchőls. Experientia 3:114-115.

Sugii, M., Suzuki, T., Nagasawa, S., and Kawashima, K. 1964. Isolation of gamma-L-glutamyl-S-allylmercapto-L-cysteine and S-allylmercapto-L-cysteine from garlic. Chem. Pharm. Bull. 12:1111-1115.

Sugimura, T., and Sato, S. 1982. Mutagens, carcinogens, and tumor promoters in our daily food. Cancer. 49:1970-1984.

Sugimura, T., Sato, S., and Wakabayashi, K. 1988. Mutagens/carcinogens in pyrolysates of amino acids and proteins and in cooked foods: heterocyclic aromatic amines. In Chemical Induction of Cancer, Structural Basis and Biological Mechanism. Eds., V. T. Woo, D. V. Lai, J.C. Arcos and M. F. Argus. pp.681-710. Academic Press, New York.

Surh, Y.J., Lee, R.C., Park, K.K., Mayne, S.T., Liem, A., and Miller, J.A. 1995. Chemoprotective effects of capsaicin and diallyl sulfide against mutagenesis or tumorigenesis by vinyl carbamate and N-nitrosodimethylamine. Carcinogenesis. 16:2467-2471.

Suzuki, T., Sugii, M., and Kakimoto, T. 1961. New gamma-glutamyl peptides in garlic. Chem. Pharm. Bull. 9:77-78.

Suzuki, T., Sugii, M., and Kakimoto, T. 1962. Gamma-L-glutamyl-S-allyl-L-cysteine, a new gamma-glutamyl peptide in garlic. Chem. Pharm. Bull. 10:345-346.

Tai, C.Y., and Ho, C.T. 1998. Influence of glutathion oxidation on thermal formation of Maillard aromas. J. Agric. Food Chem. 46:2260-2265.

Takahashi, M., Wakabayashi, K., Nagao, M., Yamamoto, M., Masui, T., Goto, T., Kinae, N., Tomita, I., and Sugimura, T. 1985. Quantification of 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) and 2-amino-3-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) in beef extracts by liquid chromatography with electrochemical detection (LCEC). Carcinogenesis 6:1195-1199.

Takahashi, M., Toyota, K., Aze, Y., Furuta, K., Mitsumori, K., and Hayashi, Y. 1993. The rat urinary bladder as a new target of heterocyclic amine carcinogenicity: tumor induction by 3-amino-1-methyl-5H-pyrido[4,3,-*b*]indole acetate. Jap. J. Cancer Res. 84:852-858.

Taylor, R.T., Fultz, E., and Knize, M.G. 1985. Mutagen formation in a model beef boiling system. Ill. Purification and identification of three heterocyclic amine mutagens-carcinogens. J. Environ Sci. Health. 20:135-148.

Terao, J., Piskula, M., and Yao, Q. 1984. Protective effect of epicatechin, epicatechin gallate, and quercetin on lipid peroxidation in phospholipid bilayers. Arch. Biochem. Biophys. 308:278-284.

Thiebaud, H.P., Knize, M.G., Kuzmicky, P.A., Felton, J.S., and Hsieh, D.P. 1994. Mutagenicity and chemical analysis of fumes from cooking meat. J. Agric. Food Chem. 42:1502-1510.

Thomson, B. 1999. Heterocyclic amine level in cooked meat and the implication for New Zealanders. Euro. J. Cancer. Prev. 8:201-206.

Troll, W., Frenkel, K., Wiesner, R. Protease inhibitors: their role as modifiers of the carcinogenic process. Adv. Exp. Med. Biol. 1986. 199:153-165.

Trompeta, V., and O'Brien, J. 1998. Inhibition of mutagen formation by organosulfur compounds. J. Agric. Food Chem. 46:4318-4323.

Tsai, S.J., Jenq, S.N., and Lee, H. 1996. Naturally occurring diallyl disulfide inhibits the formation of carcinogenic heterocyclic aromatic amines in boiled pork juice. Mutagenesis. 11:235-240.

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.

Turesky, R.J., Forter, C.M., Aeschbacher, H.U., Wurzner, H.P., Skipper, P.L., Trudel, L.J., and Tannenbaum, S.R. 1989. Purification of the food-born carcinogens 2-amino-3-methylimidazo-(4,5-*f*)quinoline and 2-amino-3,8-dimethylimidazo(4,5-*f*)quinoxaline in heated meat products by immunoaffinity chromatography. Carcinogenesis. 10:151-156.

Ueda, Y., Kawajiri, H., Miyamura, N., and Miyajima, R. 1991. Content of some sulfur-containing components and free amino acids in various strains of garlic. J. Jpn. Soc. Food Sci. Technol. 38:429-434.

Vahl, M., Gry, J., and Nielsen, P. A. 1988. Mutagens in fried pork and the influence of frying temperature. (Abstract). Mutation Res. 203:239.

Virtanen, A., Hatanaka, M., and Berlin, M. 1992. Gamma-L-glutamyl-S-n-propylcystein in knoblauch (*Allium sativum*). Suom. Kemistil. B. 35:52-58.

Virtanen, A. 1965. Studies on organic sulfur compounds and other labile substances in plants. Phytochemistry. 4:207-228.

Virtanen, A. 1969. Antimikrobielle und antithyreoide Stoffe in einigen Nahrungspflanzen. Qual. Plant. Mater. Veg. 18:8-28.

Wakabayashi, K., Takahasi, M., Nagao, M., Sato, S., Kinae, N., Tomita, I., and Sugimura, T. 1986. Quantification of mutagenic and carcinogenic heterocyclic amines in cooked foods. Develop. Food Sci. 363-371.

Wakabayashi, K., Kim, I., Kurosaka, R., Yamaizumi, Z., Ushiyama, H., Takahashi, M., Koyota, S., Tada, A., Nukaya, H., Goto, S., Sugimura, T., and Nagao, M. 1995. Identification of new mutagenic heterocyclic amine. In Heterocyclic Amine in Cooked Foods: Possible Human Carcinogens. Eds., Adamsom, R., Gustavsson, J., Ito, N., Nagao, M., Sugimura, T., Wakabayashi, K., and yamazoe, Y. pp. 197-206. Princeton Scientific Publishing Co., Princeton, NJ.

Wakabayashi, K. and Sugimura, T. 1998. Heterocyclic amines formed in the diet: carcinogenicity and its modulation by dietary factors. J. Nutr. Biochem. 9:604-612.

Wang, Y.Y., Vuolo, L.L., Spingarn, N.E., and Weisburger, J.H. 1982. Formation of mutagens in cooked foods: The mutagen reducing effect of soy protein concentrates and antioxidants during frying of beef. Cancer Lett. 16:179-189.

Wattenberg, L.W. Sparnins, V.L., and Barany, G. 1989. Inhibition of N-nitrosodiethylamine carcinogenesis in mice by naturally occurring organosulfur compounds and monoterpenes. Cancer Res. 49:2689-2692.

Wedzicha, B. 1992. Chemistry of sulphiting agents in food. Food Addit. Cont. 9:449-459.

Wei, C.I., Kitamura, K., and Shibamoto, T. 1981. Mutagenicity of Maillard browning products obtained from a starch-glycine model system. Food Cosmet. Toxicol. 19:749-751.

Weisburger, J.H. 1991. Carcinogens in foods and cancer prevention. Adv. Exp. Med. Biol. 289:137-151.

Weisburger, J.H., Nagao, M., Wakabayashi, K., and Oguri, A. 1994. Prevention of heterocyclic amine formation by tea and tea polyphenols. Cancer Letters. 83:143-147.

Weiss, R.F. 1986. Neues vom knoblauch. Arztez. Naturheilverf. 27:206-209.

Wertheim, T. 1844. Untersuchung des Knoblauchols. Ann. Chem. Pharm. 51:289-315.

Yamaizumi, Z., Shiomi, T., Kasai, H., Nisimura, 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. Mutation Res. 173:1-7.

Yen, G.C., and Chen, H.Y. 1995. Antioxidant activity of various tea extracts in relation to their antimutagenicity. J. Agric. Food Chem. 43:27-32.

Yoshida, D., and Okamoto, H. 1980. Formation of mutagens by heating creatine or amino acids with addition of fatty acids. Agric. Biol. Chem. 44:3025-3027.

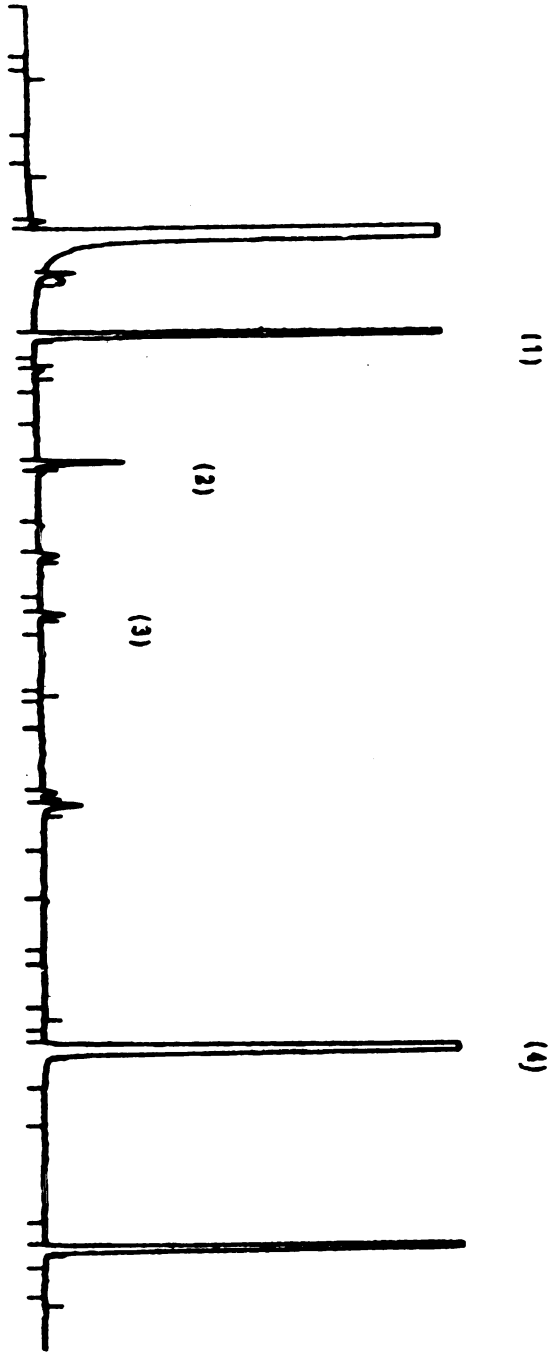
Yoshida, D., Sato, Y., and Mizusake, S. 1984. Isolation of 2-amino-3-methyl-imidazo[4,5-*f*]quinoline as a mutagen from the heated product of a mixture of creatine and proline. Agric. Biol. Chem. 48:241-847.

Yoshida, S., Kasuga, S., Hayashi, N., Ushiroguchi, T., Matsuura, H., and Nakagawa, S. 1987. Antifungal activity of ajoene derived from garlic. Appl. Environ. Microbiol. 53:615-617.

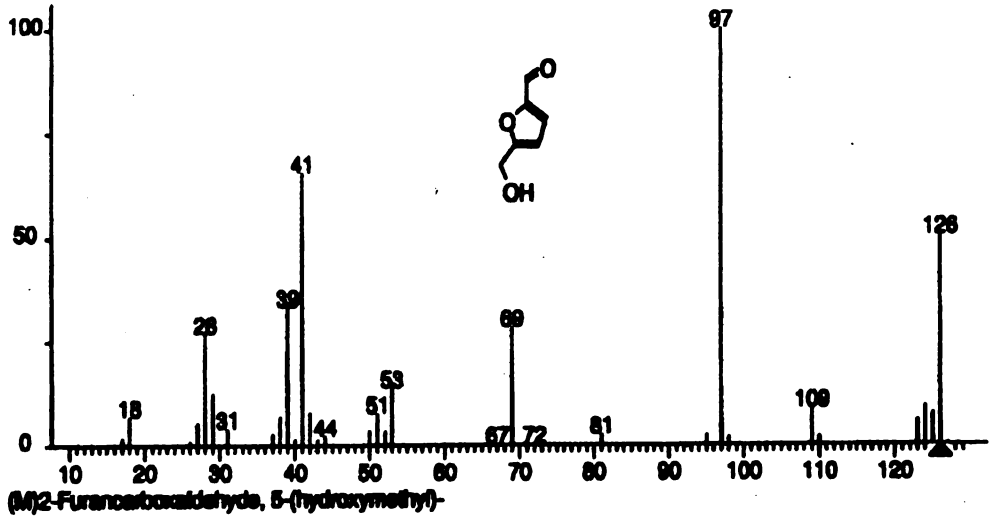
Yu, T.H., Wu, C.W., and Ho, C.T. 1994. Meat-like flavor generated from thermal interaction of glucose and alliin or deoxyalliin. J. Agric. Food Chem. 42:1005-1009.

Zhang, X.M., Wakabayashi, K., Liu, Z.C., Sugimura, T., and Nagao, M. 1988. Mutagenic and carcinogenic heterocyclic amines in Chinese cooked foods. Mutation Res. 201:181-188.

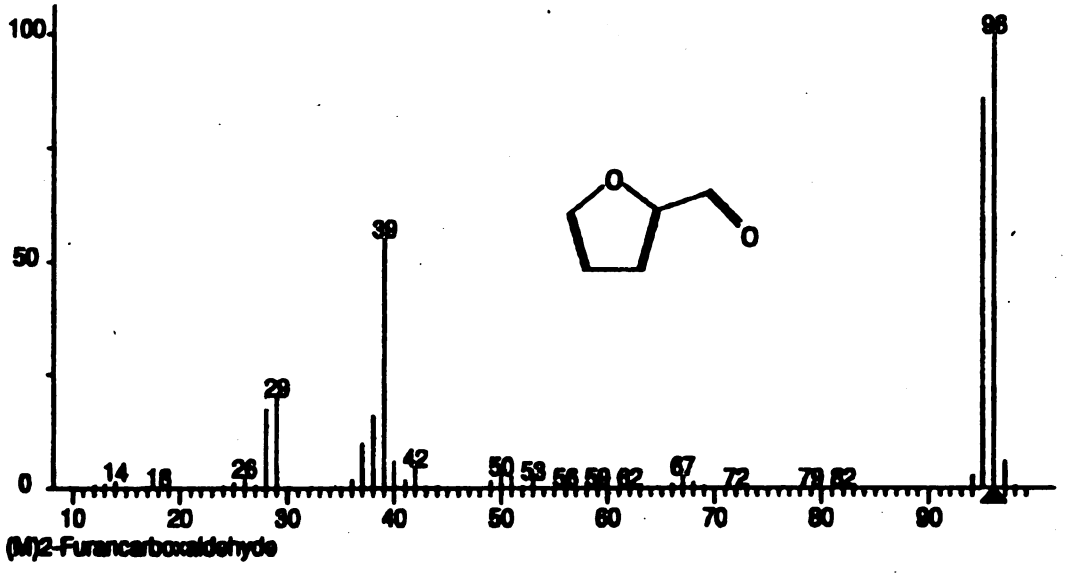
APPENDIX I



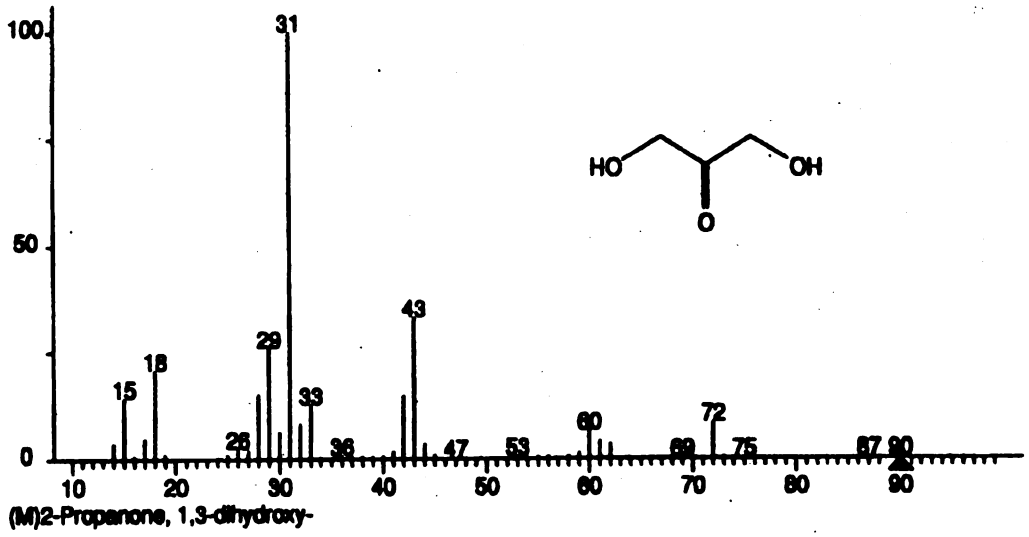
Gas chromatogram of compounds produced on heating glucose at 180C for 30 min



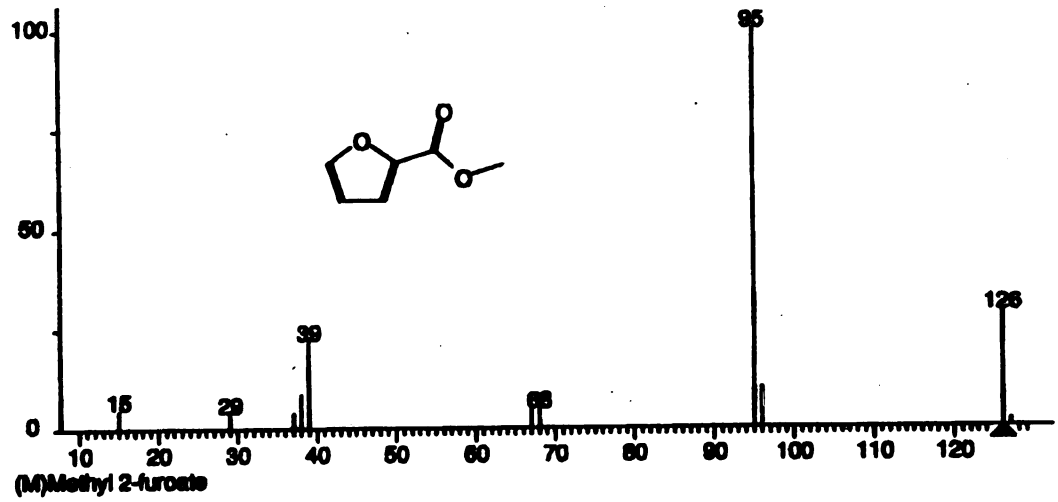
Mass spectrum of 5-(hydroxymethyl) 2-furancarboxaldehyde (Peak 1 on chromatogram).



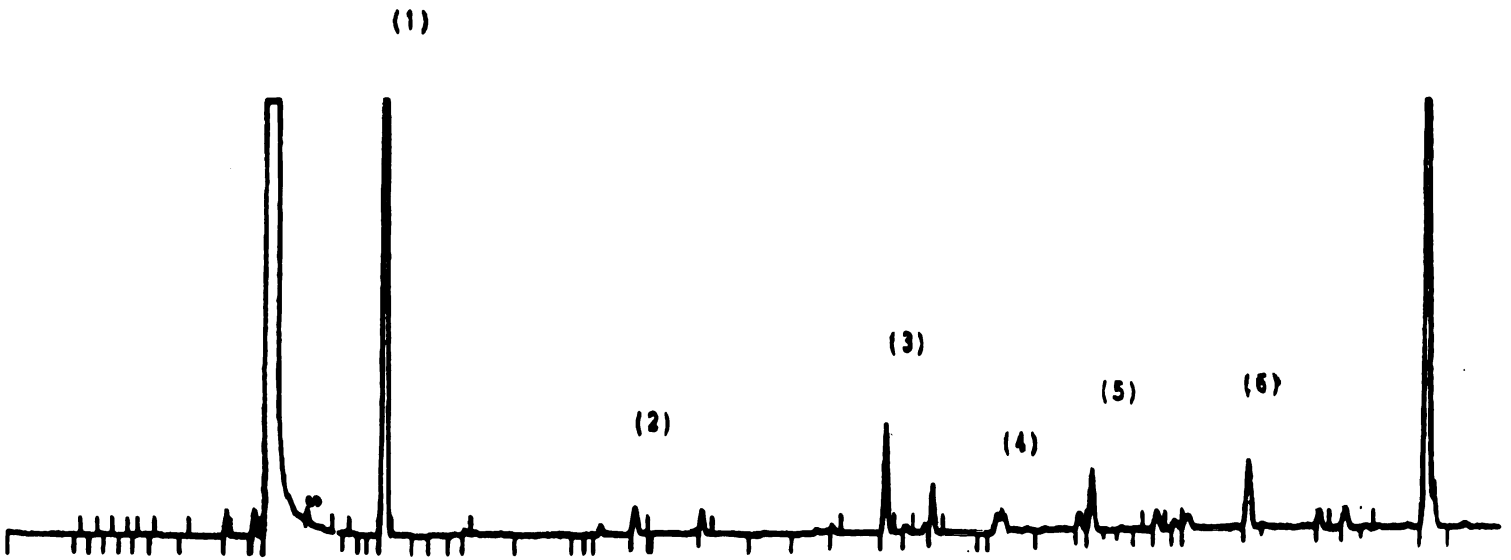
Mass spectrum of 2-furancarboxaldehyde (Peak 2 on chromatogram).



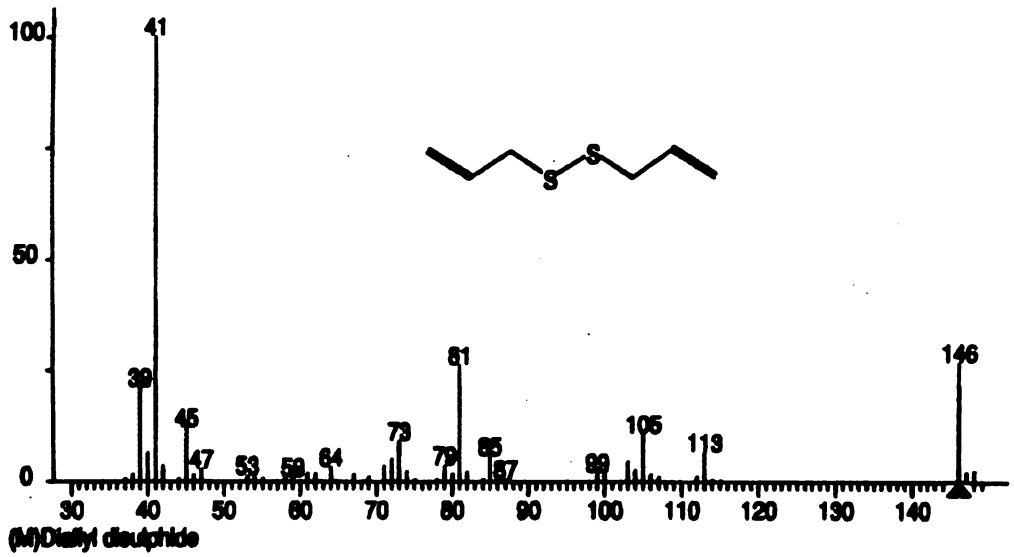
Mass spectrum of 1,3-dihydroxy 2-propanone (Peak 3 on chromatogram).



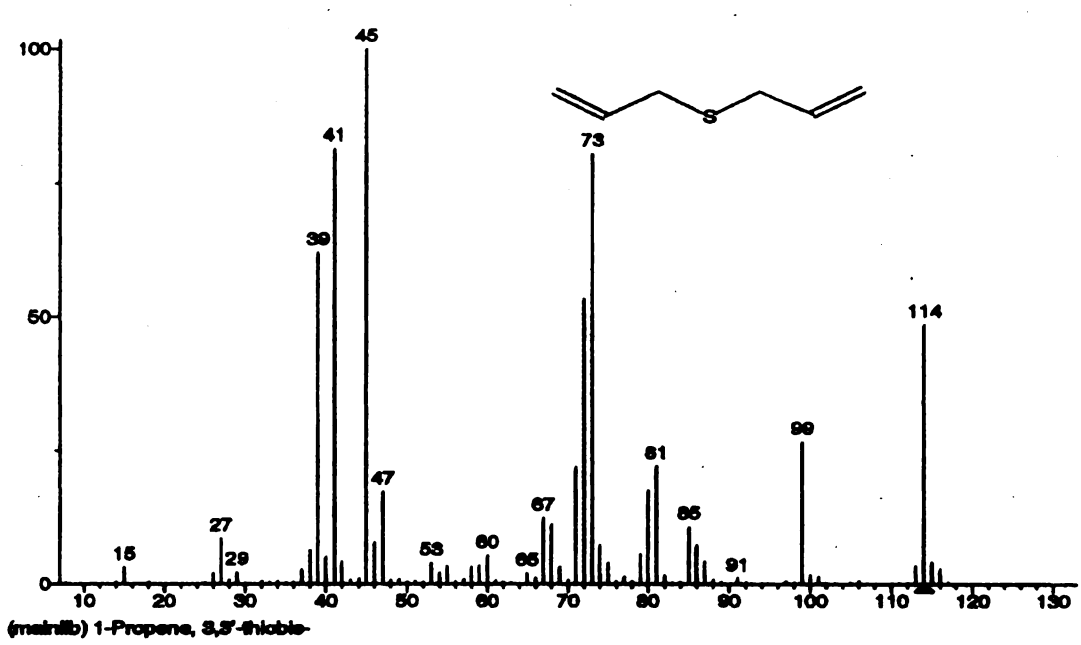
Mass spectrum of Methyl 2-furoate (Peak 4 on chromatogram).



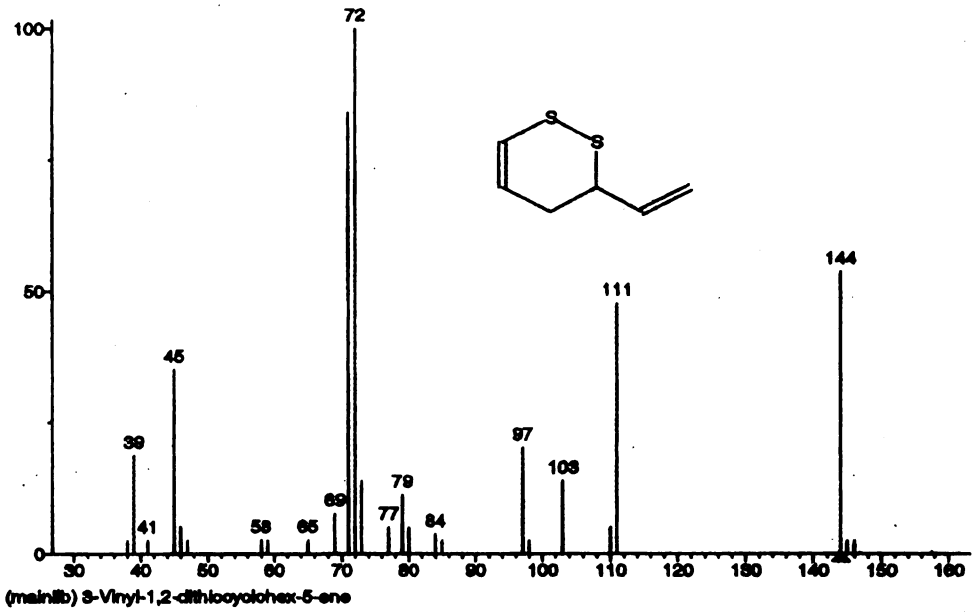
Gas chromatogram of compounds produced on heating diallyldisulfide at 180C for 30 min



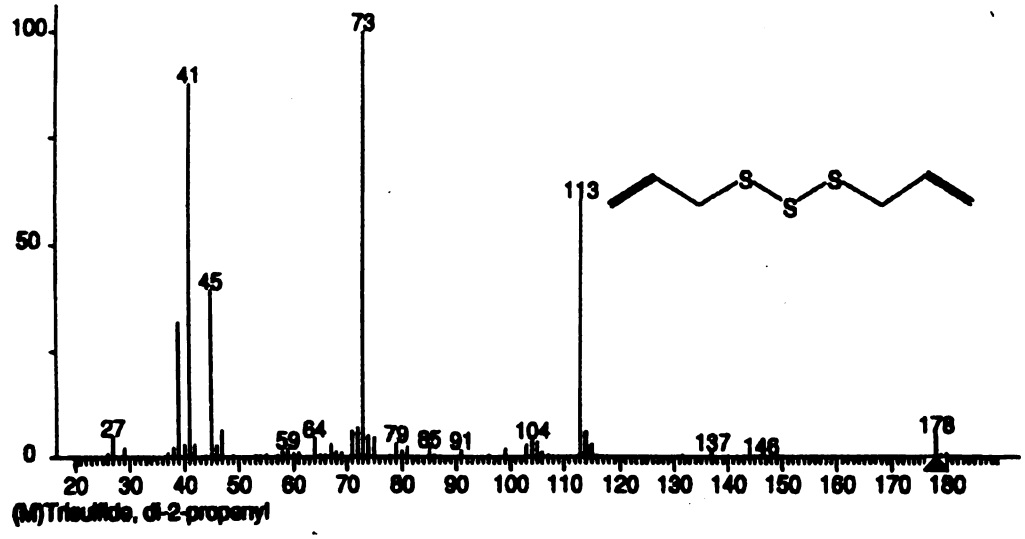
Mass spectrum of Diallyldisulfide (Peak 1 on chromatogram).



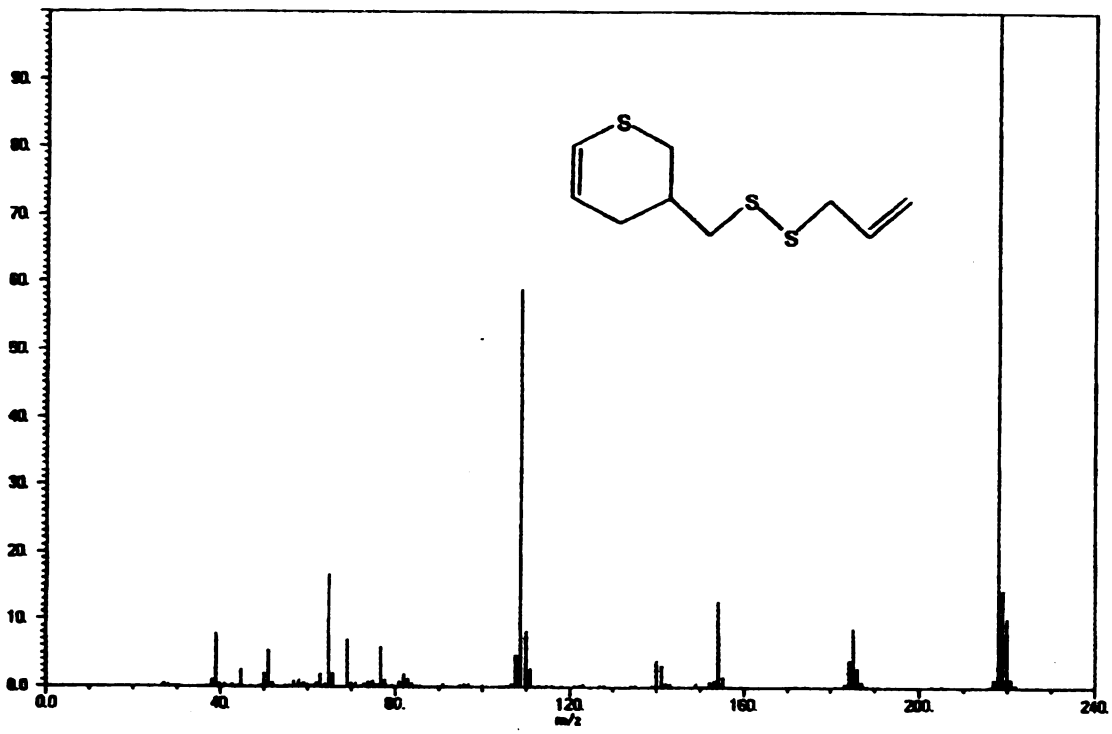
Mass spectrum of diallylsulfide (Peak 2 on chromatogram).



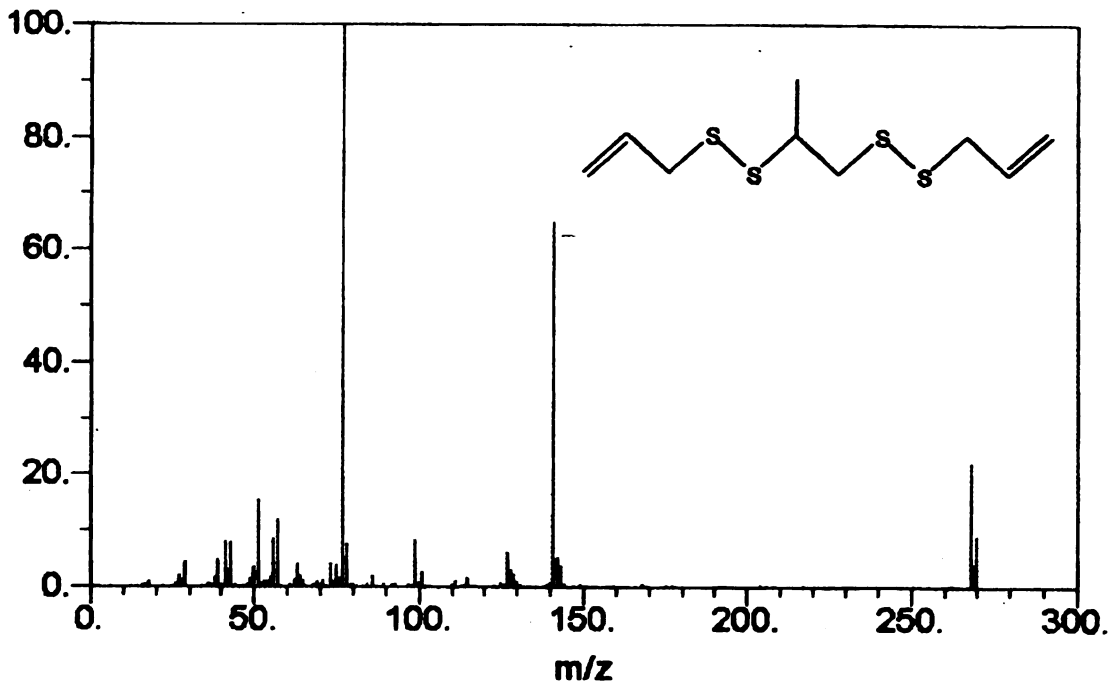
Mass spectrum of 3-vinyl-1,2-dithiocyclohex-5-ene (Peak 3 on chromatogram).



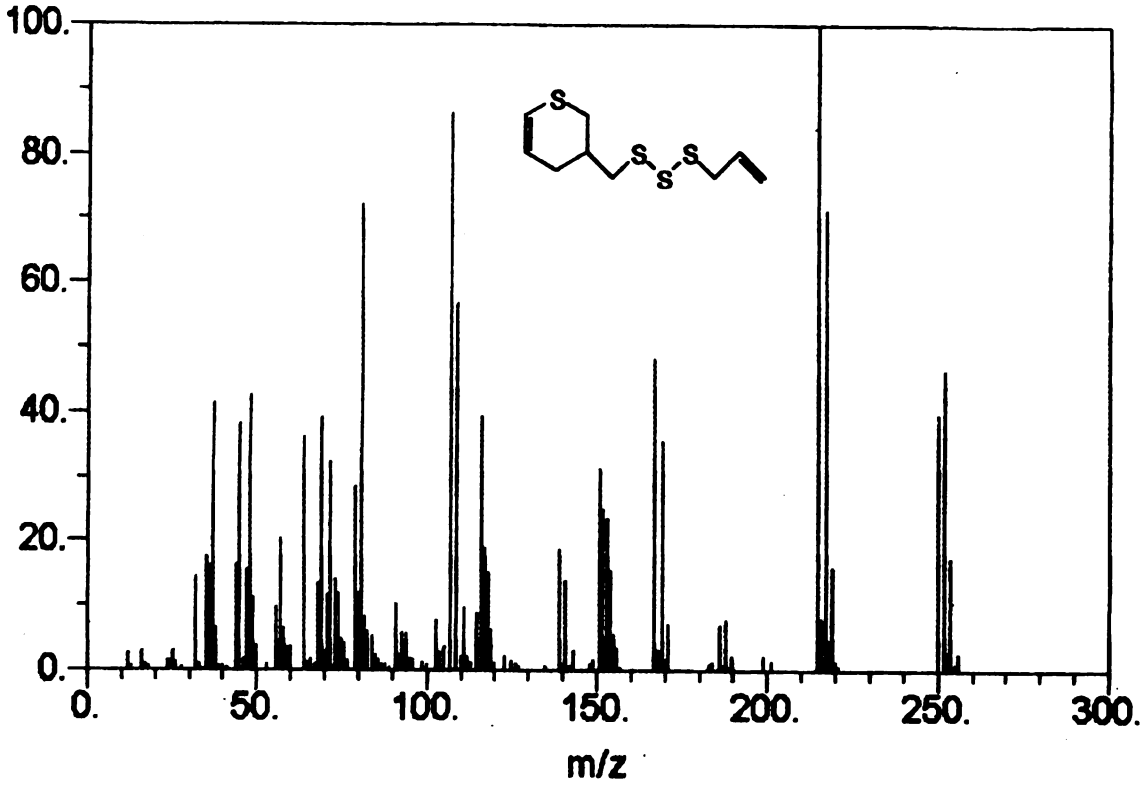
Mass spectrum of diallyltrisulfide (Peak 4 on chromatogram).



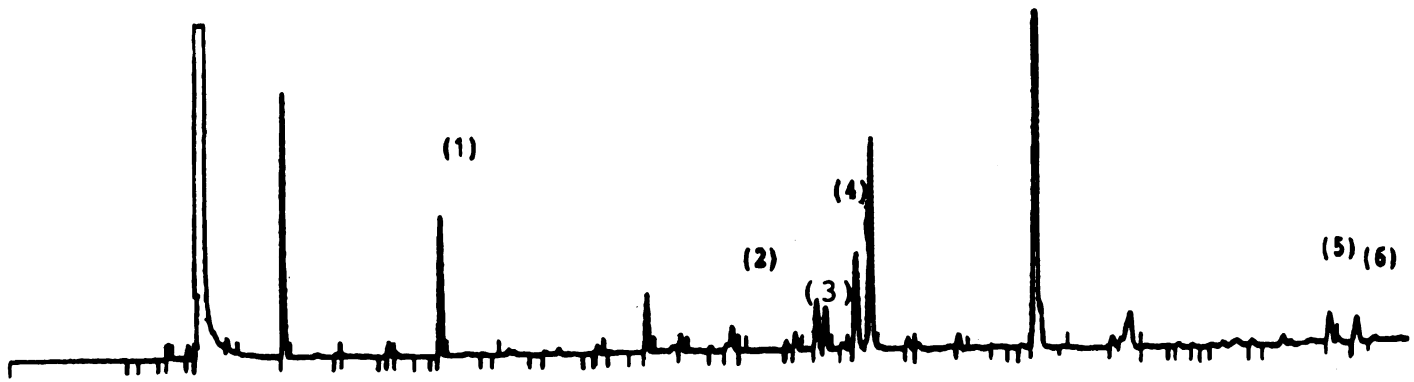
Mass spectrum of 3-(2,3-dithia-5-hexenyl)-3,4-dihydro-2*H*-thiopyran (Peak 5 on chromatogram).



Mass spectrum of 6-methyl-4,5,8,9-tetrathiadodeca-1,11-diene (Peak 6 on chromatogram).

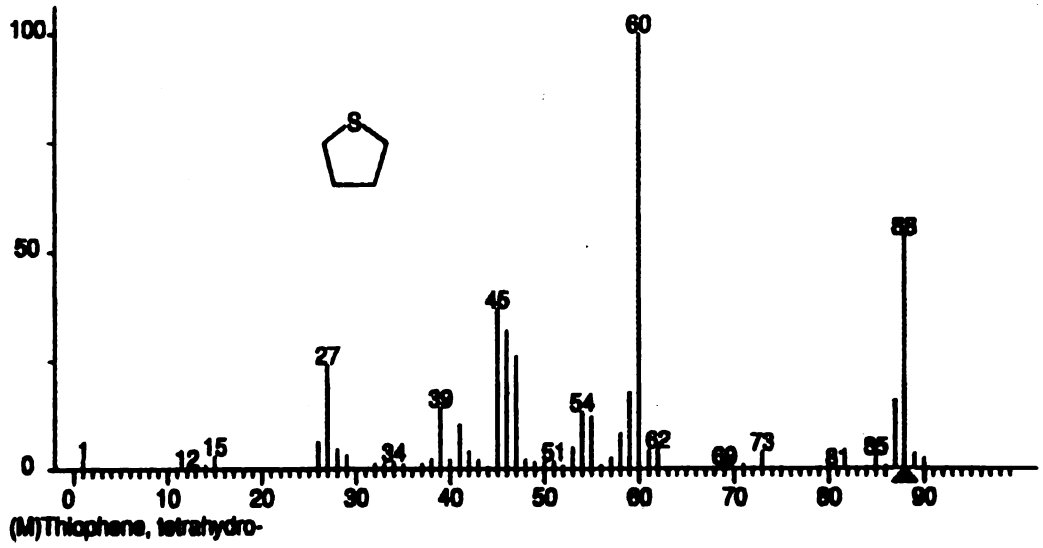


Mass spectrum of 3-(2,3,4-trithia-6-heptenyl)-3,4-dihydro-2*H*-thiopyran (Peak 7 on chromatogram).

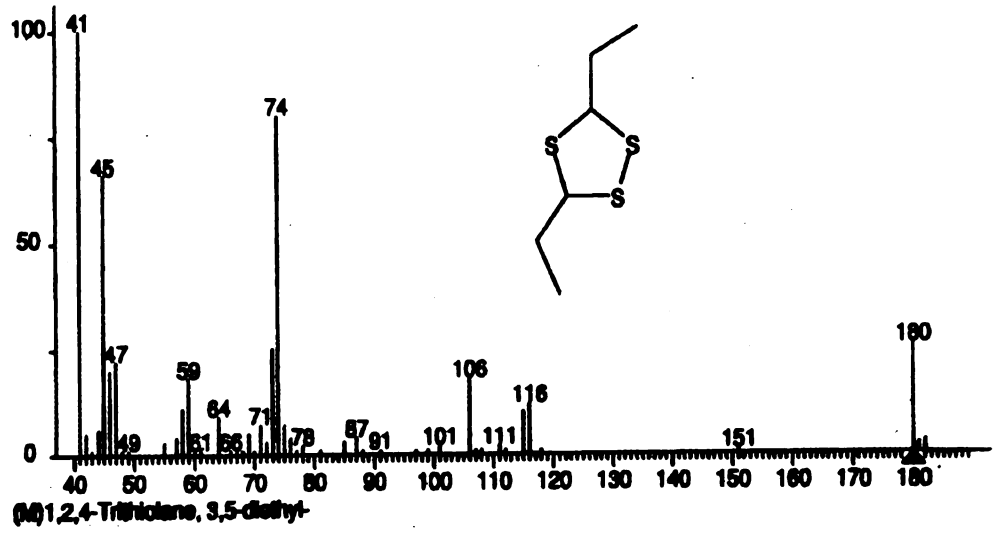


Gas chromatogram of compounds produced on heating glucose and diallyldisulfide at

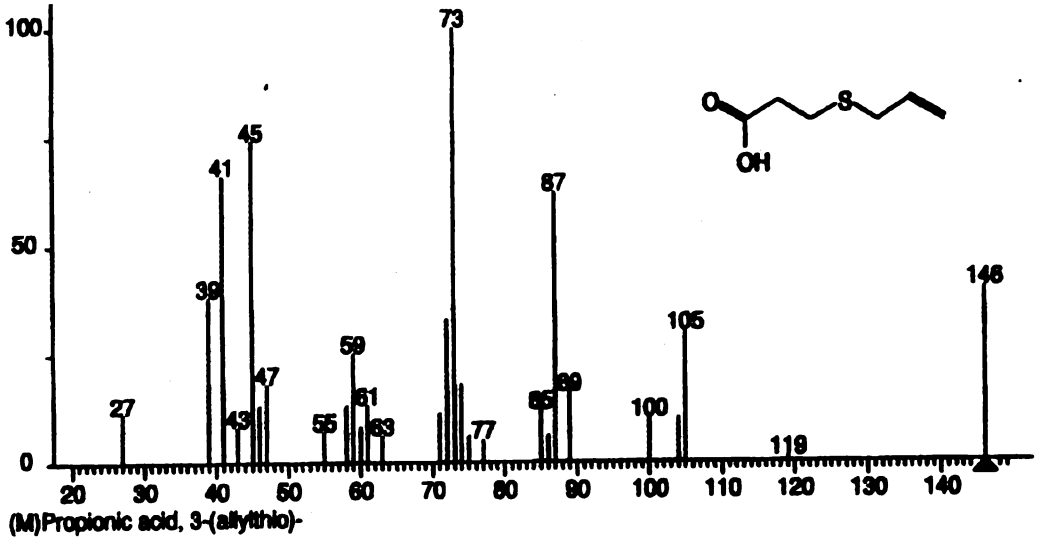
180C for 30 min



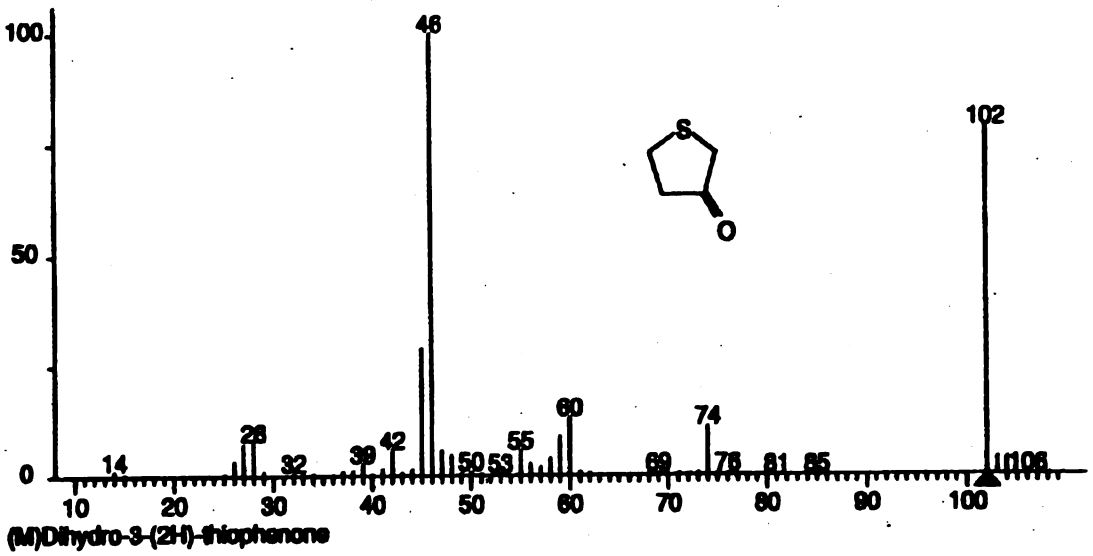
Mass spectrum of tetrahydrothiophene (Peak 1 on chromatogram).



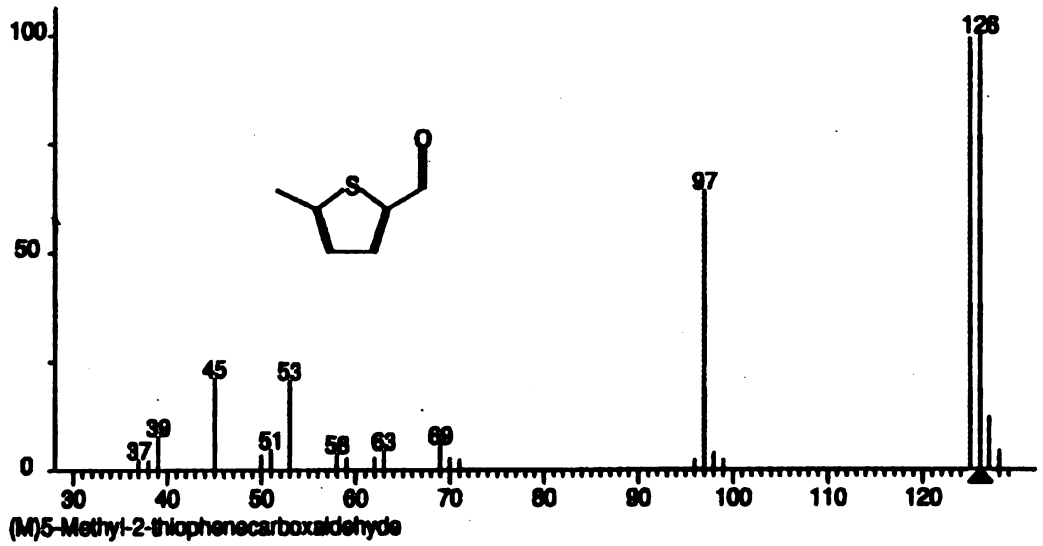
Mass spectrum of 3,5-diethyl-1,2,4-trithiolane (Peak 2 on chromatogram).



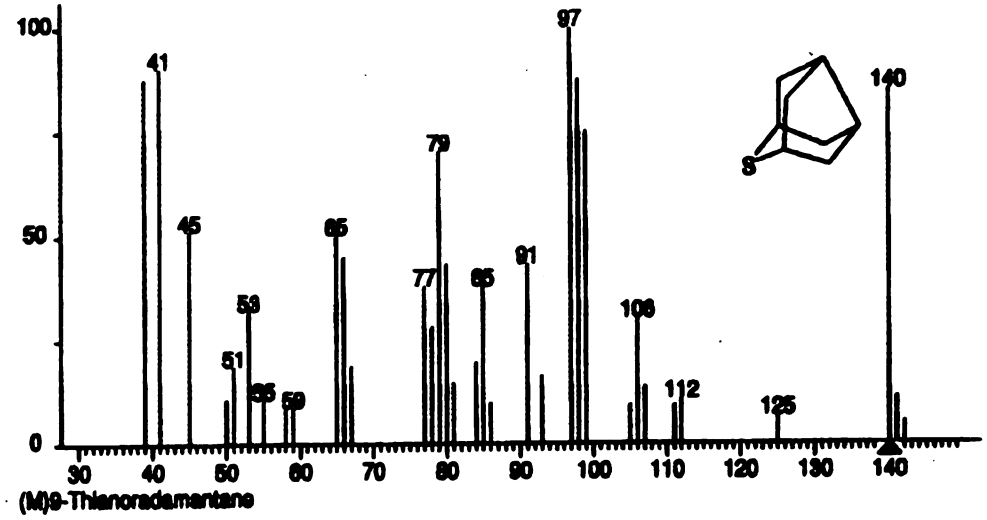
Mass spectrum of 3-(allylthio)-propionic acid (Peak 3 on chromatogram).



Mass spectrum of tetrahydrothiophene-3-one (Peak 4 on chromatogram).



Mass spectrum of 5-methyl-2-thiophene carboxaldehyde (Peak 5 on chromatogram).



Mass spectrum of 9-thianoradamantane (Peak 6 on chromatogram).