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Mutagenicity of Pyrolytic Products of Korean Animal Protein Foods by *Salmonella*/ Mammalian-Microsome Test 1. Mutagenicity of Korean-style Beef "*Bulgogi*"

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In spite of high incidences in gastrointestinal cancers in Korea, there have been few studies so far, which tried to detect or isolate the mutagenic, namely the carcinogenic substances in the Korean taste traditional foods. Among the many plausible dietary sources of carcinogens, the pyrolytic products of proteins or amino acids would be the major ones. And it is more noteworthy that the food pattern analysis showed the increasing consumption of protein foods by Korean as pyrolytic products, such as *Bulgogi* and *Kalbi, etc.* Therefore with a purpose to detect the mutagenic potential in the Korean taste protein foods, the mutagenicities of *Bulgogi* at 4 different starting temperatures of cooking were studied by the *Salmonella*/mammalian-microsome mutagenicity test, using the strains of TA98, TA100 and TA102. The organic solvents of varying polarity, were used for fractionation of samples such as fat-soluble fraction, methanol fraction and basic fraction. From a series of the experiments, the major results can be summarised as follows: 1. The analysis of temperature change during the cooking showed that the larger temperature drop was observed at the higher initial temperature state of the frying pan in pan-broiling of the meat. The degree of meat browning was relatively proportional to temperature of the cooking pan, which was most marked in the fat-soluble fraction. 2. The effect of temperature on the mutagen formation during the cooking of meat is to increase the mutagenicity of the samples with higher temperature, which was marked in *Bulgogi* on TA98 system with S9 mixture. But the mutagenicity detected by TA100 system was very high even at low temperature without S9 activation system. 3. The comparison study of the respective fractions of the cooked meat with the control of raw meat showed the notable differences. The fractions from the fresh and raw beef samples showed none of the mutagenic activity at all. The fat-soluble fraction of *Bulgogi* showed the high mutagenic activities on TA98 system with S9 mixtures, while the methanol fraction and basic fraction showed the mutagenic activities on TA100 system. But in the latter case, the S9 mixture addition caused the reduction of the mutagenic potential of those methanol and basic fractions. These data suggested that the fat-soluble fraction of meat has the mutagenicity of indirect-acting, frame-shift type, while the basic fraction has the mutagenicity of direct-acting, base-pair substitution type. And these results indicated that the mutagens of meat have been newley formed through cooking process. In conclusion, this study shows that the Korean taste protein foods have a large potential of mutagenicity and the traditional methods of cooking, including cooking temperature, seasoning and choice of cooking apparatus cause the difference in the mutagen formation.

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INTRODUCTION

Our daily diet contains numerous mutagenic substances (Sugimura, 1982). Of those that have been isolated and identified, several are natural ingredients of the food materials, while others are artificially added or contaminated materials (Ames, 1983). Moreover, recently, it was shown that some of dietary mutagens are formed as a result of the cooking process, especially of protein-rich foods (Spingarn & Weisburger, 1979; Spingarn *et al.*, 1980). Since Sugimura *et al.* (1979) reported bacterial mutagens on the surface of charred meat and fish, in excess of what could be accounted for benzo(a) pyrene content, several mutagenic heterocyclic amines have been isolated from pyrolyzed amino acids, proteins and proteinous food (Sugimura & Sato, 1983; Nagao *et al.*, 1977; Sugimura, 1985), Actually, the protein foods, represented as muscle foods, are one of the best sources of nutrients for human growth and maintenance, being especially rich in high-quality protein, vitamins and minerals. However, the advent of the mutagenic potential by cooking of the protein foods warned us to be prudent. In spite that the epidemiological study in Korea indicated the high incidences of stomach cancer and hepatoma (Ministry of Health, 1986), suggesting the deep relationship with Korean traditional dietary habit, there have been a few study which elucidate the mutagenic potentials in the Korean foods (Park, 1980; Park *et al.*, 1980; Kimm *et al.*, 1982). And the recent trend of increasing protein-food consumption in Koreans attracted our attention to study the mutagenic potential, present in *"Bulgogi"*, the most favorite and common protein foods of Koreans. *Bulgogi* is a kind of beef-derived dishes, spiced with various vegetables and soy sauce *etc.* We usually take it after pan-broiling. It was reported that the formation of mutagenic potential in the protein foods was affected by cooking temperature (Commoner *et al.*, 1978; Spingarn & Weisburger, 1979; Pariza *et al.*, 1979; Dolara *et al.*, 1979). However, there have been few reports on the formation of mutagenic potentials from *Bulgogi* in relation with cooking process, such as heating temperature, cooking utensils or additive spices. For the analysis of mutagens, *Salmonella*/mammalian microsome test of Ames is most popular in the aspect of being easy, economic, rapid and very sensitive. And the strains of *S. typhimurium his*$^+$ auxotrophs can be reverted by the different modes of mutation, such as base-pair substitution, frame-shift or oxidative mutation (Levin *et al.*, 1982; Ames *et al.*, 1975). Therefore, the mutation analysis by Ames test made it possible to identify the mode of mutation by the specific chemicals. In the present study, we intended to determine the degrees and conditions of mutagen formation in *Bulgogi* cooking by use of *Ames* test.

METHODS AND MATERIALS

Reagents

The chemicals were purchased from the following sources; L-histidine-HCl, ampicillin, crystal violet, glucose-6-phosphate monosodium, NADP, 4-nitroquinoline-N-oxide(NQO)

and N-methyl-N'-nitro-N'-nitrosoguanidine (MNNG) from Sigma Chemical Co. (St. Louis, Mo. U.S.A.): Bacto-agar, Bacto-tryptone, Bacto-yeast extract from Difco. Co. (Detroit, U.S.A.): Aroclor 1254 and dimethylsulfoxide(DMSO) from Wako Chemical Co. (Wako, Japan). And other reagents of analytical grade were obtained from the available commercial sources.

Checking-out procedure of tester strains

Strains of TA97, TA98, TA100, TA102 and TA104 were kindly donated by Professor Ames (Univ. of California, Berkeley, Calif. USA). And those tester strains were checked-out as the recommended procedures for *his*$^+$ auxotrophy, antibiotic resistence, *rfa* character of membrane, UV and/or radical sensitivity (Ames *et al.*, 1975;Levin *et al.*, 1982).

Table 1. Genotypes of strains used in the present experiment*

Strains	Histidine mutation	LPS[a]	Repair[b]	R-factor[c]
TA 98	his D 3052	*rfa*	*uvrB*	+R
TA 100	his G 46	*rfa*	*uvrB*	+R
TA 102	his G 428(PAQ1)	*rfa*	+	+R

a; Lipopolysaccharide barrier, in deep rough character *(rfa):* LPS removed down to the ketooxyoctanoate lipid core

b; Deletion through Δ *uvrB* includes the nitrate reductase *(chl)* and biotin genes *(bio)*

c; Ampicillin resistance factor

* Adapted from Maron and Ames (1983).

Preparation of S-9 mix

The microsomal fractions to be used for mutagen activation were prepared by intraperitoneal injection of Aroclor 1254 (500 mg/kg rat weight in corn oil) into Sprague-Dawley male rats. The supernatant of 9,000 x g centrifugation of the liver homogenate was distributed into 1 ml aliquots and preserved in liquid-nitrogen tank until assay. When required, S-9 fraction was mixed with the following chemicals per ml and used as "S-9 mix": S-9 fraction (40 μl), $MgCl_2$ (8 μmol), KCl (33 μmol), glucose-6-phosphate (5 μmol), NADP (4 μmol) and sodium phosphate buffer (pH 7.4, 100 μM).

Quality control of Ames test

For the quality control purpose, we checked daily the mutagenic potentials, induced by MNNG, direct-acting carcinogen and NQO, a indirect-acting carcinogen with or without S-9 mix, on each tester strain as TA98, TA100. And to TA102, mutagenic effect by mitomycin C was monitored for oxidative mutation. For mutagen analysis of each sample, the respective dose response on mutagenic activity was determined. The test was carried out after the standard procedure, recommended by Ames (Ames *et al.*, 1975).

Determination of cooking temperature

The temperature of the cooking frying pan was determined by attaching the chromel/alumel alloy conducting device on the center of the frying pan. And the temperature change of the frying pan during the cooking was monitored.

Preparation of Bulgogi Sample

Samples of *Bulgogi* were prepared after the standard cooking procedure as follows; one hour soaking of sliced beef after mixing with various spices and broiling on teflon-coated frying pan over gas burner. The recipe of *Bulgogi* was as follows; 2½ Table spoon (T.S.) of sugar, soy sauce 5 T.S., sesame salt 1½ T.S., sesame oil 1½ T.S., 2 roots of onion, 1 clove of garlic, ½ part of pear, dash of black pepper per 600g of beef, Each two hundred gram of *Bulgogi* was heated two minutes and turned upside-down, followed by further two more minute cooking at varying starting temperatures of frying pan such as 100°C, 150°C, 200°C and 250°C. These cooked *Bulgogi* samples were collected, lyophilized and preserved at deep freezer (Revcon, -70°C) until extraction.

Extraction procedure

For the extraction of mutagenic substances of different chemical nature, we followed Övervik's procedure (Övervik *et al.*, 1984). As summarized in Fig. 1, each one gram of

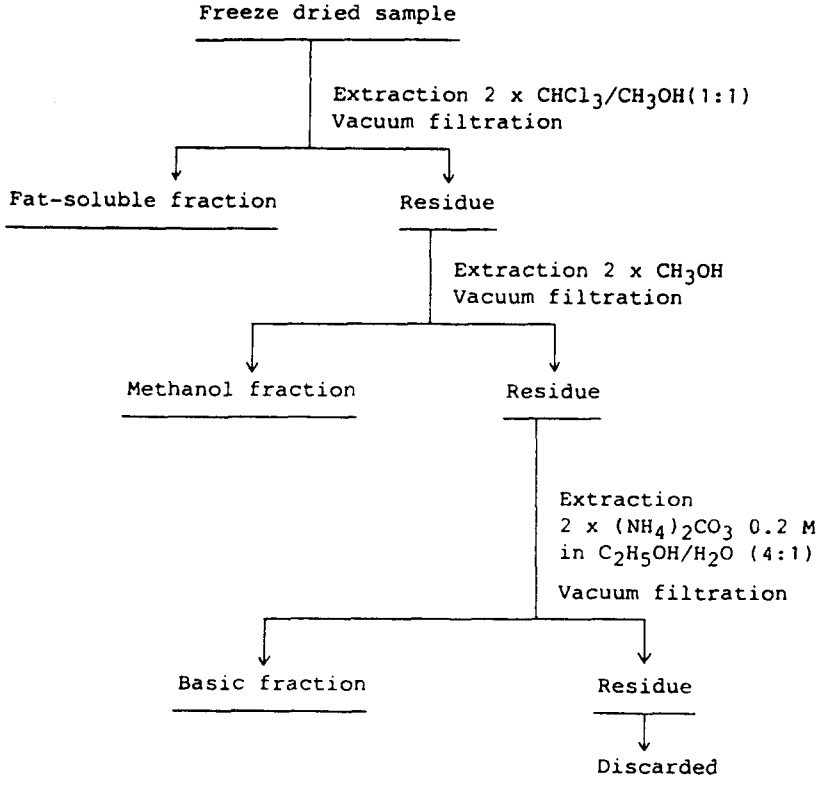

Fig. 1. Scheme for extraction of mutagenic activity from meat samples

freeze-dried sample was vacuum-filtered after overnight mixing and stirring with 5 ml of chloroform/methanol (1:1 v/v) solution. The filtrate of twice extraction was stored overnight at cold room and again vacuum-filtered. The filtrate was concentrated by evaporization of solvents and dissolved in DMSO, which was used as "Fat-soluble fraction". The residue of chloroform/methanol extraction was subjected to further twice extraction with methanol. The extracted filtrate was concentrated, dried and dissolved in DMSO, which was regarded as "Methanol fraction". The final residue of methanol treatment was extracted twice with 0.2 M ammonium carbonate in ethanol-water (4:1 v/v) solution. The final extract was filter-dried and dissolved in DMSO, which was used as "Basic fraction".

Monitoring of browning of samples

The browning of sample from each extraction procedure was monitored by measurement of absorbance change at 430 nm with Spectronic 21.

RESULTS

Effect of starting temperature on cooking condition

The temperature of cooking frying pan was monitored by the central attachment of chromel/alumel alloy heat-conducting device. The practical cooking

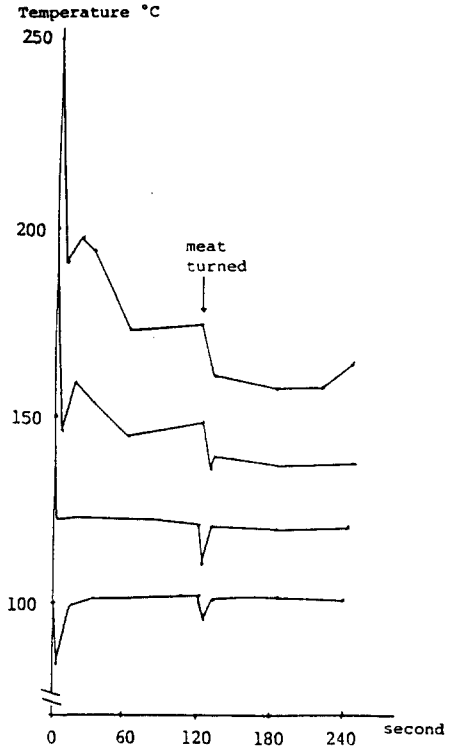

Fig. 2. Time dependence of temperature of frying pan for different starting temperatures

temperature of *Bulgogi* was changed by application of the samples on the frying pan, probably due to the contained water in the *Bulgogi.* The effect of contained water on temperature-fall was manifested by the increase of frying pan after 220 second cooking at 250°C, when most of water in *Bulgogi* might have been evaporized (Fig. 2). The fall of the actual cooking temperature was most marked at the highest starting-temperature of cooking. The lower the starting temperature, the less fall of cooking temperature was observed. By turning upside down of *Bulgogi,* further decrease of cooking temperature was detected.

Effect of temperature on browning of samples

Each sample of fat-soluble", "methanol", and "basic" fraction from *Bulgogi,* prepared by different starting temperatures was checked for its absorbance at 430 nm before concentration (Fig. 3). As the increase of starting temperature for cooking, the absorbance of "fat-soluble" fraction was increased. But in cases of "methanol" and "basic" fractions, there were no significant change in absorbance in relation with alteration of cooking condition. These results suggested that most of browning substances in cooking of meat were fat-soluble.

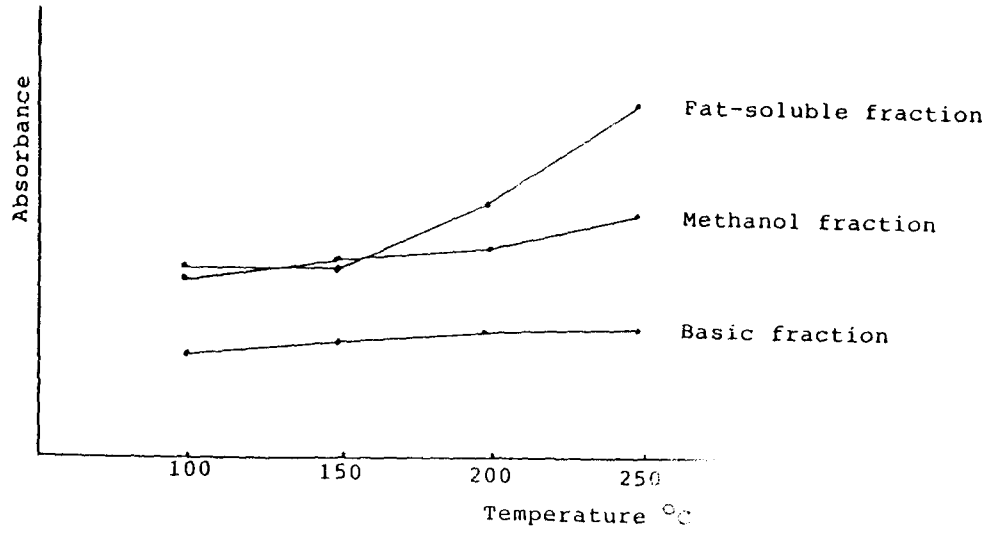

Fig. 3. Temperature dependence of absorbance at 430 nm for 3 fractions

Mutagenicity of Bulgogi

Mutagen detection with TA98.

TA 98 tester strain of Ames test is known to detect the frame-shift type mutagens. *Bulgogi* samples were tested for mutagenicity with TA98 after varying extraction in function of dose and cooking temperature. The results were summarized in Table 2. All the data were converted to the number of revertent colonies, induced by the testing substances, by miligram equivalent of dry weight sample per plate. The most marked mutagenic activities were detected in fat-soluble fraction rather than in methanol or basic fraction. By increase of starting cooking temperature, the more mutagens of indirect-acting type in the fat-soluble fraction were formed up to 200°C, which required microsomal activation for mutagenic activities. But further increase of cooking temperature up to 250°C increased the formation of direct-acting mutagens but decreased the formation of indirect-acting mutagens. These results suggested that, the lower the cooking temperature was, the less the mutagens were formed, which could be detected by frame-shift type mutagen detection system, TA98.

Mutagen detection with TA100.

TA100 tester strain of Ames test detects mostly the base-pair substitution type mutagens. In the present experiment, all the *Bulgogi* samples of varying dose and cooking temperature were extracted into "fat-soluble", "methanol" and "basic" fractions by difference of organic solvent solubility. The results of screening of mutagen formation in the *Bulgogi* samples by TA100 system were summarized in Table 3. The mutagen formation of base-pair substitution type in *Bulgogi,* detected by TA100, was marked in basic fraction or methanol fraction rather than in fat-soluble fraction. And the increase of cooking temperature over 150°C did not accompany the increase of mutagen formation. The methanol fraction showed rather high mutagenic activities of dose response in low cooking temperature, lower than 150°C, requiring microsomal activation for their base-pair substitution type activities. The basic fractions of varying *Bulgogi* samples showed the high mutagenic activities at low cooking temperature condition. Most of the mutagens in the basic fractions were of direct-acting type, which did not require microsomal activation. But samples from hot cooking condition over 200°C showed the less mutagen content.

Mutagen detection with TA102

TA102 tester strain of Ames test detects the mutagens of oxidative radical generation. The results of mutagen screening with TA102 on varying *Bulgogi* samples were summarized in Table 4. In most of the samples, the more of oxidative mutagens of direct acting type, which did not require microsomal activation for mutagenic activities, were detected with the increase of cooking temperature. The fat-soluble fraction from 200°C showed the higher mutagenic

Table 2. Mutagenicity of extracts from *'Bulgogi'* on TA 98 of *Salmonella typhimurium* at different starting temperature

Starting temp. (°C)	Extracts[a]	S9[b]	mg equiv. of dry wt. sample/plate								
			10	20	50	100	150	200	250	300	400
100	1	+	0[c]	0	6	9	20	31	42	12	
		-	0	0	0	10	16	14	5	0	
	2	+	1	4	8	23	28	34	-[d]	15	19
		-	7	5	-	25	16	25	-	42	28
	3	+	0	12	11	22	-	14	-	0	0
		-	0	0	0	0	-	14	-	17	37
150	1	+	4	9	17	31	167	-	286	14	
		-	2	1	9	25	116	110	76	46	
	2	+	0	4	18	32	48	46	-	75	45
		-	0	0	14	16	15	17	-	58	25
	3	+	7	9	72	10	2	0			
		-	4	4	29	6	0	5			
200	1	+	0	0	12	214	256	1860	1290	16	
		-	0	0	59	112	193	35	32	0	
	2	+	7	4	18	31	29	-	13		
		-	0	8	12	19	39	5	0		
	3	+	0	36	25	21	0	0			
		-	8	37	32	14	0	0			
250	1	+	12	15	20	24	42	34	14		
		-	0	6	31	221	615	22	0		
	2	+	7	12	17	24	17	16	0		
		-	1	13	1	20	32	6	0		
	3	+	18	59	21	34	6	10	0		
		-	17	2	18	59	15	11	0		

a: 1, Fat-soluble fraction; 2, Methanol fraction ; 3, Basic fraction

b: +; Tested in the presence of S9 mixture
-; Tested in the absence of S9 mixture
S9 mixture (1 ml) ; composed of rat liver S9 fraction (40 μl), $MgCl_2$ (8 μmol), KCl (33 μmol), glucose-6-phosphate (5 μmol), NADP (4 μmol) and sodium phosphate (100 μmol, pH 7.4)

c: number of revertant colonies per plate, subtracted of the number of spontaneous reversion

d: -; not detected

Table 3. Mutagenicity of extracts from '*Bulgogi*' on TA 100 of *Salmonella typhimurium* at different starting temperature

Starting temp. (°C)	Extracts[a]	S9[b]	mg equiv. of dry wt. sample/plate								
			10	20	50	100	150	200	250	300	400
100	1	+	22[c]	35	44	72	79	108	37	0	
		-	15	27	45	41	58	47	0	0	
	2	+	23	25	57	82	-[d]	142	-	578	926
		-	0	0	0	45	-	137	-	208	392
	3	+	0	71	71	67	-	412	-	508	0
		-	0	118	92	510	-	1794	-	80	0
150	1	+	30	19	32	69	105	101	76	46	25
		-	11	47	54	110	55	85	-	-	36
	2	+	0	5	31	62	129	161	-	1038	1139
		-	0	9	23	45	134	166	-	527	488
	3	+	44	100	126	157	111	125	-	85	
		-	22	162	653	699	73	0	-	0	
200	1	+	0	6	43	22	0				
		-	30	46	22	59	0				
	2	+	20	10	30	109	151	185	190	29	
		-	31	89	80	112	143	149	178	40	
	3	+	0	32	81	139	210	105	42	0	
		-	-	61	70	106	43	41	-	65	
250	1	+	4	-	32	64	52	79	80	113	
		-	28	-	64	82	95	89	0	0	
	2	+	21	39	88	48	193	190	185	25	
		-	27	58	104	133	215	183	145	0	
	3	+	36	49	99	131	136	178	83	87	
		-	0	21	113	194	-	199	0	0	

a: 1, Fat-soluble fraction ; 2, Methanol fraction; 3, Basic fraction
b: +; Tested in the presence of S9 mixture
-; Tested in the absence of S9 mixture
S9 mixture (1 ml) ; composed of rat liver S9 fraction (40 μl), $MgCl_2$ (8 μmol), KCl (33 μmol), glucose-6-phosphate (5 μmol), NADP (4 μmol) and sodium phosphate (100 μmol, pH 7.4)
c: Number of revertant colonies per plate, subtracted of the number of spontaneous reversion
d: -; not detected

Table 4. Mutagenicity of extracts from *'Bulgogi'* on TA 102 of *Salmonella typhimurium* at different starting temperature

Starting temp. (°C)	Extracts[a]	S9[b]	mg equiv. of dry wt. sample/plate							
			10	50	100	150	200	250	300	400
100	1	+	0[c]	9	50	0	0	0	0	
		-	0	104	201	-[d]	402	25	28	
	2	+	60	-	159	-	242	-	0	10
		-	0	-	92	-	202	-	13	16
	3	+	0	32	23	-	68	-	0	0
		-	0	0	0	-	0	-	0	0
150	1	+	24	102	10	0	0			
		-	0	96	73	30	46			
	2	+	6	78	260	61	80	133	-	75
		-	41	108	180	147	194	123	-	15
	3	+	0	127	191	52	0	0		
		-	0	239	365	26	0	0		
200	1	+	0	29	69	-	277	-	136	
		-	0	156	1934	85	49	-	30	
	2	+	0	77	112	-	30	0		
		-	96	88	132	-	8	0		
	3	+	13	62	0	0				
		-	0	0	0	0				
250	1	+	24	73	5	-	144	0		
		-	25	-	107	167	193	0		
	2	+	85	64	109	-	0			
		-	132	186	204	-	0			
	3	+	0	0	0	0	0			
		-	0	0	0	0	0			

a: 1, Fat-soluble fraction ; 2, Methanol fraction ; 3, Basic fraction

b: +; Tested in the presence of S9 mixture
-; Tested in the absence of S9 mixture
S9 mixture (1 ml); composed of rat liver S9 fraction (40 μl), $MgCl_2$ (8 μmol), KCl (33 μmol), glucose-6-phosphate (5 μmol), NADP (4 μmol) and sodium phosphate (100 μmol, pH 7.4)

c: Number of revertant colonies per plate, subtracted of the number of spontaneous reversion

d: -; not detected

Table 5. Mutagenicity of extracts from *'Bulgogi'* and fresh meat on 2 strains of *Salmonella typhimurium* at different starting temperature

Extract fractions	Starting temp. (°C)	g equiv. of dry wt. sample			
		TA98 +S9[a]	TA98 -S9[b]	TA100 +S9	TA100 -S9
Fat-soluble	Fresh meat	0[c]	0	0	0
	100	168	83	540	388
	150	1144	550	703	1100
	200	9300	1293	860	590
	250	281	4120	372	636
Methanol	Fresh meat	0	0	0	0
	100	170	138	2315	980
	150	320	191	2847	1739
	200	310	261	925	712
	250	240	214	1293	1440
Basic	Fresh meat	0	0	0	0
	100	220	92	1676	8970
	150	1440	580	1570	6990
	200	1800	1850	1407	1060
	250	340	590	890	1940

a : +S9 ; Tested in the presence of S9 mixture

b : -S9 ; Tested in the absence of S9 mixture
S9 mixture(1 ml) ; composed of rat liver S9 fraction (40 μl), $MgCl_2$ (8 μmol), KCl (33 μmol), glucose-6-phosphate (5 μmol), NADP (4 μmol) and sodium phosphate (100 μmol, pH 7.4)

c : Number of revertant colonies per plate, subtracted of the number of spontaneous reversion

activities, detected by TA102, while methanol fractions of varying cooking temperature sources showed the relatively high mutagenic activities.

Mutagenicity of Bulgogi broiled on the cast iron griddle

The mutagenicity of extracts from *Bulgogi* gas-broiled on the cast iron griddle of Korean traditional type was analyzed by TA98 and TA100 tester strains with or without S-9 mix. The results were summarized in Table 6. In this experiment, the monitoring and exact adjustment of cooking temperature were not carried-out, because of open gas-broiler. But this is one of the most traditional type of *Bulgogi* cooking in Korea. The samples were collected and extracted under the same procedure. The mutagenic activities of this type *Bulgogi* were most prominent in the fat-soluble fraction, detected by TA98

with S-9 activation, which suggested the high induction of indirect-acting mutagenic potential of frame-shift type. The results were compatible with the data of *Bulgogi* by frying-pan cooking in the present experiment (Table 2 and 6). But the mutagens of base-pair substitution type were rarely detected by TA100 system in the cast iron griddle-broiled *Bulgogi* samples.

Table 6. Mutagenicity of extracts from *'Bulgogi'* gas-broiled on the cast iron griddle

Strains	Extract fractions	S9[a]	mg equiv. of dry wt. sample/plate						
			10	50	100	150	200	300	400
TA 98	Fat-soluble	+	7[b]	51	77	-[c]	1519	1431	
		-	2	-	151	-	339	335	
	Methanol	+	0	0	0	48	197		
		-	0	39	182	43	0		
	Basic	+	0	55	171	0	0		
		-	0	3	47	0	-	82	0
TA100	Fat-soluble	+	0	0	61	-	158	72	
		-	0	0	0	0	0	0	
	Methanol	+	0	0	49	-	9	0	
		-	0	0	41	0	0	0	
	Basic	+	0	0	79	0	0	0	0
		-	0	0	0	0	0	6	25

a : + ; Tested in the presence of S9 mixture
- ; Tested in the absence of S9 mixture

S9 mixture (1 ml) ; composed of rat liver S9 fraction (40 μl), $MgCl_2$ (8 μmol), KCl (33 μmol), glucose-6-phosphate (5 μmol), NADP (4 μmol) and sodium phosphate (100 μmol, pH 7.4)

b : Number of revertant colonies per plate, subtracted of the number of spontaneous reversion

c : - ; not detected

DISCUSSION

The validation of Ames test for mutagen screening was well established. And recently more new tester strains were added to the standard procedure for improvement in detectability and sensitivity (McCann *et al.*, 1975; Maron

& Ames, 1983). Among them, for the detection of frame-shift type mutagen, the revertent *his*$^+$ colony fomation in TA98 was recommended, while for base-pair substitution type mutagen, TA100 system was suggested, and recently for the detection of oxidative mutagens, such as formaldehyde, glyoxal, mitomycin C, bleomycin, X-ray and UV-ray, TA102 tester strain was added to the standard procedure (Levin *et al.*, 1982). And in test with all these tester strains, the mammalian microsomal activation system was recommended to use if necessary, thereby imitating *in vivo* metabolic change of the mutagenic substances. Formation of many mutagenic substances by cooking of muscle foods was already detected with the present Ames test. Many different heterocylic amines with high mutagenic activities, found in charred fish or meat, were isolated and identified to be in two major different classes: namely, imidazoquinoline (IQ) class and non-imidazoquinoline (non-IQ) class, according to the sensitivity to nitrite treatment (Sugimura, 1985). IQ class of mutagens includes 2-amino-3-methylimidazo [4,5-f] quinoline (IQ), 2-amino-3,4-dimethylimidazo [4,5-f] quinoline (MeIQ), 2-amino-3,8-dimethylimidazo [4,5-f] quinoxaline (MeIQx), 2-amino-3,4,8-trimethylimidazo [4,5-f] quinoxaline (4,8-DiMeIQx), and 2-amino-3,7,8-trimethylimidazo [4,5-f] quinoxaline (7,8-DiMeIQx), *etc*, while non-IQ class includes 2-amino-6-methyl-dipyrido [1,2-a:3′,2′-d] imidazole (Glu-P-1), 2-amino-dipyrido [1,2-a:3′,2′-d] imidazole (Glu-P-2), 3-amino-1,4-dimethyl-5H-pyrido [4,3-b] indole (Trp-p-1), 3-amino-1-methyl-5H-pyrido [r,3-b] indole (Trp-P-2), 2-amino-α-carboline (AαC), 2-amino-3-methyl-α-carboline (MeAαC), 2-amino-3-methyl-α-carboline (MeAαC), and 2-amino-2-ethyl-α-carboline (EtAαC), *etc* (Sugimura *et al.*, 1977; Takeda *et al.*, 1977, 1978; Kosuge *et al.*, 1978; Yamazoe *et al.*, 1980; Hosaka *et al.*, 1981; Yamamoto *et al.*, 1978; Yamaguchi *et al.*, 1979, 1980; Yoshida & Matsumoto, 1978). Most of these mutagens were reported to be formed by direct heating of the protein or amino acids over 300°C. And the mutagenicity of these cooking process-related mutagens were much more stronger than most of other natural mutagens, such as benzopyrene, aflatoxin B_1 and N-nitrosodimethylamine (Sugimura, 1982). And the possible role of these mutagens in the food as the carcinogen was also well documented in the animal experiments (Takayama *et al.*, 1977, 1984 a,b; Tohda *et al.*, 1980; Matsukura *et al.*, 1981; Ohgaki *et al.*, 1984 a,b). It would be admitted, therefore, that the mutagens in the foods, whether natural ingredients, contaminants or newly generated products by cooking procedure, are most responsible for the carcinogenesis of gastrointestinal tract.

For the detection of mutagens, formed during the cooking procedure, the extraction with methylene chloride was originally applied. But inactivation and destruction of mutagens during this extraction procedure had substituted the methods with consecutive extraction with chloroform/methanol, methanol and ammonium salt solution (Övervick *et al.*, 1984). The fat-soluble fraction, extracted with chloroform/methanol would include the non-polar substances such as glycerides, phospholipids and heterocyclic amines, *etc.* while the methanol fraction would contain the residual phospholipid and rather neutral compounds. But the basic fraction, finally extracted with 0.2 M ammonium carbonate solution in ethanol water, might have many polar mutagens, such as

various nitrosamines. Therefore, by application of this serial extraction method, many different types of mutagens could be separated.

The high incidence of stomach cancer and hepatoma among Korean population attracted us to screen the mutagens in the Korean traditional or taste foods. Actually, we reported the high content of mutagens, readily activated by simple nitrosation, among Korean taste marine foods (Park *et al.*, 1980). But so far, there have been few study, which screened the mutagenic potential in relation with cooking procedure for Korean taste foods, in spite of the high consumption of *Bulgogi,* Korean-style beef, among Korean. The formation of mutagens in *Bulgogi* during cooking would be clearly expected from the study on roasted beef, chicken, bacon *etc.* And in the study of mutagen formation during cooking, the cooking temperature condition was suggested to be most important (Bjeldanes *et al.*, 1982; Krone & Iwaoka, 1984; Miller & Buchanan, 1983). Therefore, in the present study, we tried to elucidate the condition of mutagen formation in *Bulgogi* cooking under the purpose to establish a recommended cooking procedure of least mutagen formation. The variable of the present study in *Bulgogi* cooking procedure was the starting temperature. We prepared the samples by four minute cooking on frying pan with turning upside-down once at the respective starting temperature of 100°C, 150°C, 200°C and 250°C. Actual surface temperature of *Bulgogi* was relatively lower than the starting temperature, probably by cooling effect of the contained water; namely, approximately 175°C for 250°C starting temperature, 150°C for 200°C group, 130°C for 150°C group and 100°C for 100°C group. The lower the starting temperature was the less fall of practical surface temperature of meat was observed (Fig. 2). The degree of formation of charred substances by cooking of *Bulgogi* was determined by the change of absorbance at 430 nm. The marked increase of absorbance was noticed in the fat-soluble fraction, extracted with chloroform/methanol as a function of starting temperature of cooking *Bulgogi,* which indicated that the heat-dependent mutagens were the substances of non-polar type mutagens (Fig. 3).

The results of mutagen screening test for *Bulgogi* samples could be summarized briefly as follows: with the increase of starting cooking temperature, the more mutagens of frame-shift type with non-polar character, which required microsomal activation were formed (Table 2), and in case of base-pair substitution type mutagens, the more mutagens of direct-acting type were formed at the lower starting temperature of cooking (Table 3). And for the case of oxidative mutagen formation, it was evident that the more mutagens of direct-acting type were formed with the increase of starting cooking temperature (Table 4). In case of oxidative mutagen detection, the data on dose-response effects were not definite, probably because the stability of oxidative radicals generated was poor. Therefore, we summarized the results from TA98 and TA100 assay for the convenience of comparison by converting the data into numbers of revertent colony formation induced by gram equivalent of dry weight sample (Table 5). The results indicated the increase in fat-soluble mutagen formation of indirect-acting frame-shift type, requiring microsomal activation with increase of cooking temperature and the increase of base-pair

substitution type mutagen formation of direct-acting type, polar in nature at low cooking temperature (Table 5). But in case of uncooked fresh meat, none of mutagenic activities were detected in any type of extraction. These results clearly suggested the formation of mutagens during the cooking procedure by heat, compatible with the other studies (Park *et al.*, 1980; Dolara *et al.*, 1979). The high induction of mutagen formation from cooking of beef, pork, bacon and hamburgher at 150°C-200°C were reported, which was compatible with the present result from *Bulgogi* study (Gocke *et al.*, 1982; Lin *et al.*, 1982; Overvick, 1984; Miller & Buchanan, 1983). The decrease of mutagens detected by TA98, 100 and 102 over 200°C were not clearly explained, but we could assume that the stability of the mutagens might be influenced by the high temperature. These results of mutagen formation, mostly in relation with increase in cooking temperature warned us to reduce the temperature of cooking foods (Commoner *et al.*, 1978a, b; Spingarn & Weisburger, 1979; Pariza *et al.*, 1979; Miller & Buchanan, 1983 a,b). And the differences in mutagen formation were observed by the change of cooking utensils from frying pan to cast-iron griddle. Although the general pattern of mutagen formation was rather similar, the synthesis of direct-acting type mutagens was different in some sense (Table 6). The reduced formation of mutagens from beef cooking was observed in steaming, high-pressure cooking or microwave ovening in contrast to the high induction of mutagen formation by frying and roasting (Miller & Buchanan, 1983a, b). And the difference of surface material of cooking utensils such as aluminum, iron, stainless steel in comparison with china or enamelized ware made distinction in mutagen formation (Bjeldanes *et al.*, 1983).

From these results, it could be concluded that the modification of cooking procedure in case of *Bulgogi* would significantly reduce the formation of mutagens through adjustment of cooking temperature and utensils. But we did not disregard the importance of spice effect on mutagen formation from *Bulgogi,* which should be studied in detail some time. And we acknowledge the significance of mutagen screening research on other Korean taste protein foods: chicken, pork, *Kalbi* and varying fishes in varying condition of cooking procedure.

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Salmonella/mammalian microsome실험법에 의한 육류식품 열분해산물의 돌연변이 유발능에 관한 연구

1. 한국 '불고기'의 돌연변이능

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굽기 시작시 후라이팬의 온도변화에 따른 불고기 시료의 여러가지 유기용매 추출물을 대상으로 Ames법을 행한 결과는 다음과 같다.

1. 굽기 시작시 후라이팬의 온도가 높을수록 고기표면의 온도 변동이 컸고 갈색화 현상이 더 많이 일어났다.
2. 불고기에 있어서 TA98에 대한 돌연변이 유발능은 200℃까지 온도의 상승에 따라 증가하였고, 이러한 변이능은 S9 mix.에 의하여 활성화됨으로써 간접작용 돌연변이 유발성 물질의 존재를 시사하였다. TA100에 대한 돌연변이 유발능은 100℃와 150℃ 등의 낮은 온도에서도 비교적 높았으며 'methanol fraction'을 제외하고는 S9 mix.에 의한 영향이 적어 직접작용 돌연변이 유발성 물질도 생성되었음을 보여주었다.
3. 불고기의 'fat-soluble fraction'은 TA98에 대한 돌연변이 유발능이 높았으며, 그 돌연변이 유발능은 S9 mix.에 의하여 활성화가 증진되었다. 동 시료의 'methanol fraction'에서는 TA100에 의해 검출되는 물질이 많이 존재했으며 이러한 물질은 S9 mix.에 의해 활성화 되었다. 'Basic fraction'에는 TA100에 의해 검출되는 돌연변이 유발성 물질이 많이 존재하였으나 250℃를 제외하고는 S9mix.에 의해 오히려 돌연변이 유발능이 감소하였다. 따라서 'fat-soluble fraction'에는 간접작용 위치이동 돌연변이 유발성 물질이 많이 존재하고, 'basic fraction'에는 직접작용 돌연변이 유발성 물질이 많이 존재하는 것으로 나타났다.

이와같은 실험결과로 미루어 보아 우리나라에서 널리 이용되는 불고기는 가열조리에 의하여 돌연변이 유발성 물질이 형성된다고 하겠다. 이러한 돌연변이 유발능은 가열온도에 의해 크게 영향을 받으므로 육류식품을 조리할 때는 낮은 온도에서 단시간 조리하는 것이 바람직할 것으로 사료된다.