Mutagen Formation in Heated Wheat Gluten, Carbohydrates, and Gluten/Carbohydrate Blends

Mendel Friedman,* Robert E. Wilson, and Irving I. Ziderman†

Western Regional Research Center, U.S. Department of Agriculture—Agricultural Research Service, 800 Buchanan Street, Albany, California 94710

Gluten, carbohydrates, and gluten/carbohydrate blends were heated in a simulation of low-moisture baking and assayed by the Ames *Salmonella* his-reversion test in order to evaluate the formation of mutagenic browning products. An aqueous acetonitrile extract of heated gluten was highly mutagenic when assayed with *Salmonella typhimurium* strain TA98 with metabolic activation. Weak mutagenicity was also observed with strains TA100 and TA102. Gluten heated in a vacuum oven yielded less extract than did the air-baked protein, but its mutagenic activity (revertants per milligram of extract) was similar. Baked D-glucose, maltose, lactose, sucrose, wheat starch, potato amylose, cellulose, microcrystalline hydrocellulose, sodium ascorbate and L-ascorbic acid, sodium (carboxymethyl)cellulose, or [(hydroxypropyl)methyl]cellulose were moderately mutagenic in strain TA98 with microsomal (S9) activation and were weakly mutagenic without microsomal activation in strains TA100, TA102, and TA1537. Heated blends of gluten with 20% of these carbohydrates were also mutagenic, but the total activity recovered did not exceed levels of the individual ingredients baked separately. Maillard-type melanoidins prepared from L-lysine and D-fructose were very weakly active with strain TA98 but were mutagenic without S9 activation in strains TA100, TA102, and TA2637. Possible sources of mutagens, the significance of these findings for food safety, and possible approaches to minimize mutagen formation are discussed.

Heat-induced nonenzymatic browning occurs in most foods. Such browning reactions often lead to the formation of antinutritional and toxic compounds (Fujimaki et al., 1986). Mutagen formation in baked products such as cookies and bread has been reported by Pariza et al. (1979) and Van der Hoeven (1982). Mutagenic products in cooked protein-rich foods are formed by several mechanisms, including carbohydrate caramelization, protein pyrolysis, amino acid/creatinine reactions, and amino–carbonyl (Maillard) reactions, in which free amino groups condense with reducing sugars to produce brown melanoidins, furans, carbolines, and a variety of other heterocyclic amines (Mauron, 1981; Hayashi and Shibamoto, 1984; Hargraves and Pariza, 1984; Taylor et al., 1986; Powrie et al., 1986; Fujimaki et al., 1986; Sugimura et al., 1986; Friedman and Cuq, 1988; Hatch et al., 1988; Miller, 1988; Cuzzoni et al., 1989). Amino acid–reducing sugar mixtures have been widely used to study mutagen formation. Less frequently, nonenzymatic browning of some foods also may occur by chemical reaction of acidic and neutral amino acid residues with nonreducing sugars and even with polysaccharide carbohydrates (Leszkowiat et al., 1990). These precursors are in fact much more abundant in foods than the reactants required for classical Maillard reactions. Such an alternative source of food browning would involve chemical mechanisms quite different from the amino–carbonyl reaction, and correspondingly different mutagens may be formed.

Our recent studies (Ziderman and Friedman, 1985; Ziderman et al., 1989) have shown that, in simulated crust baking of gluten, nonnutritive fiber (cellulose) reacts more vigorously than reducing sugars such as D-glucose, D-fructose, lactose, or maltose, which themselves have a reactivity similar to that of nonreducing sucrose and starches. Generally, these reactions are exothermic and cause the internal temperature of the substrate to exceed that of the baking oven. Since the depletion of gluten L-lysine residues was greater with nonreducing sucrose or cellulose than with the reducing sugars, and since the initial step in the classical Maillard reaction is postulated to involve the interaction between an NH_2 group of lysine and the carbonyl group of a reducing sugar (Mauron, 1981; Friedman, 1982), the Maillard reaction is not the only one involved in such heat-induced reactions. The non-Maillardian reactions also depleted the content of other amino acids to a degree similar to the lysine depletion (Friedman et al., 1987).

We now report studies on the mutagenicity of extracts of these heated protein-rich foodstuffs. Commercial gluten was selected as the main subject of this study because of its importance as a source of dietary protein in baked foods.

MATERIALS AND METHODS

Materials. The materials used and the procedure for simulated crust baking have been described in detail previously (Ziderman and Friedman, 1985). Maltose monohydrate was from Fisher Scientific (Chicago, IL). Other sugars and vitamin C, all chemically pure, were from ICN Nutritional Biochemical (Cleveland, OH). The sucrose was powdered in a Wiley mill (1-mm screen). Cellulose (Alphacell nonnutritive bulk), hydrocellulose (microcrystalline hydrolyzed Alphacell), and wheat starch were from ICN Nutritional Biochemicals; potato starch (Type II practical grade) was from Sigma Chemical Co. (St. Louis, MO). [(Hydroxypropyl)methyl]cellulose (HPMC) was Dow Methocell K4M premium grade (Midland, MI), and sodium (carboxymethyl)cellulose (CMC) was Hercules high-viscosity cellulose gum type 7HF (Wilmington, DE). Wheat gluten (water, 9%; free lipids, 2%; total lipids, 8%; water-saturated butanol

† Visiting Scientist at WRRC. Permanent address: Israel Fiber Institute, Ministry of Industry and Trade, P.O. Box 8001, 92 080 Jerusalem, Israel.

Table I. Mutagenicity of Heated Gluten in *S. typhimurium* Strains TA98, TA100, TA102, and TA1537[a,b]

	TA98				TA100				TA102				TA1537			
	heated		unheated		heated		unheated		heated		unheated		heated		unheated	
	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
							Gluten Extract (MGE)									
100	166**	54	46	19	200	153	100	99	584	402	591	420	14	17	10	6
20	335**	36	54	29	251**	87	153	121	611	400	573	417	28*	10	8	7
5	141**	33	50	31	159	121	140	103	569	370	643*	402	17[c]	12	12	10
0	43	24	43	24	142	117	142	117	554	403	554	403	10	10	10	10
x	14.22	0.26			5.61	0.41			2.86	0.13			0.85	0.07		
p	0.0001	0.082			0.0002	0.030			0.080	0.64			0.0006	0.044		
							Baked Gluten (Milligrams per Plate)									
2.67	164*	26			219	134			545	313						
0	68	46			171	168			559	417						
							Gluten Extract (Vacuum Baked; MGE)									
100	339**	37														
20	85*	29														
5	45	32														
0	37	22														
x	3.07	0.11														
p	0.0001	0.23														

[a] Symbols: x = slope (revertants/MGE); p = statistical significance of the slope; single and double asterisks, marked values that exceed control mean plus LSD 0.01 and control mean plus LSD 0.001, respectively. [b] As with data for some of the other tables, in some cases there is a degradation in response at the highest dose level. Therefore, the 100-MGE dose level was deleted prior to regressions for the baked gluten extract, +S9 treatment. [c] Single plates only; remaining values represent means of duplicate plates. Composite positive control values for all tables: TA98, 0.15 μg of aflatoxin (+S9), 726 → 2000 revertants per plate (r/p); TA100, 0.15 μg of aflatoxin (+S9), 1513 → 2000 r/p; TA102, 30 μg of danthron (+S9), >1200 r/p; 0.1 μg of streptonigrin (-S9), 731 → 1500 r/p; TA1537, 15 μg of emodin (+S9), 447 r/p; 10 μg of 9-aminoacridine (-S9), 1327 → 2000 r/p; TA2637, 100 μg of 9-aminoacridine (-S9), >2000 r/p.

extractables, 13%; ash, 0.9%; total carbohydrates, 11%; starch, 6%; amino acid content, 76%) was from U.S. Biochemical Corp. (Cleveland, OH).

Baking Procedures. Gluten, carbohydrates, and gluten/carbohydrate blends (4:1, w/w, air-dry basis, 8% moisture, 200 g) were baked at 215 °C for 72 min, in a forced-draft convection oven (Ziderman and Friedman, 1985). The baked products were then ground in a Wiley mill.

Extraction Procedure. The procedure was adapted from Bjeldanes et al. (1984). To 20 g of sample in a 250-mL centrifuge flask was added 200 mL of 9:1 acetonitrile/H_2O solution, and the contents were stirred for 5 min. The bottle was then centrifuged for 10 min at 9000 rpm and the supernatant filtered through a No. 509 Eton-Deikman fluted filter paper. Next, the volume was reduced to about 20 mL on Buchi Rotovapor evaporator at no more than 40 °C. This process was repeated four times with the pellet in the centrifuge tube. The combined extracts were again reduced in volume to about 40 mL, and the concentrated extract was then transferred to a preweighed 100-mL round-bottom flask. Next, the sample was completely dried on the evaporator and freeze-dried. The flask was weighed to determine yield and then stored frozen.

Mutagenicity Assays. Mutagenicity assays were carried out with use of the *Salmonella typhimurium* his-reversion assay described by Maron and Ames (1983). The standard top-agar incorporation method without preincubation was employed. S9 was prepared from Aroclor-1254-induced adult male Sprague–Dawley rats obtained from Simonsen Laboratories, Gilroy, CA. S9 mix contained 0.1 mL of S9/1 mL of mix. Dried extracts of baked samples were dissolved or suspended in dimethyl sulfoxide (DMSO), with heating to 45 °C if necessary, and were added to top agar in a volume of 0.1 mL of DMSO. Doses of extract are expressed in the tables as milligram equivalent weight of original extracted material (MGE). Unextracted baked samples were suspended in DMSO and were added to top agar in a volume of 0.1 mL. These values are expressed as milligram per plate. Colonies were counted after 48 h at 37 °C either by manual means or with an Artek Model 980 colony counter (Artek Systems Corp., 170 Finn Court, Farmingdale, NY) at a threshold setting of 0.2 mm. Positive responses were verified in at least one duplicate experiment, except where noted in the tables.

Statistical Methods. Analyses of variance were run within strain, baking treatment, and sample with use of the SAS General Linear Models Procedure (SAS GLM) (SAS, 1987). Least significant difference (LSD) comparisons with the controls in Tables I, III, and V were based on the LSMEANS PDIFF option or P value differences among means (SAS, 1987). SAS GLM was also used for regressions by making dose a continuous variable and estimating linear slopes using ESTIMATE statements. Values of log transformation of dose for regressions were not used on these data as the responses are mostly linear on the transformed scale. LSD's for Tables III and V are based on pooled, between-replicate variance estimates within strain, except for strains TA100 and TA102 for Table III. Due to heterogeneity of variance in these cases, variances were estimated separately for controls and treated samples, and the LSD's were computed with use of the Satterthwaite (1946) approximation.

RESULTS

After being heated in a convection oven, commercial wheat gluten was assayed in the Ames Test with *S. typhimurium* strains TA98, TA100, TA102, and TA1537. No activity above the spontaneous revertant frequency was observed without S9 microsomal activation with the possible exception for TA98 (Table I). At a dose of 2.7 mg/plate of unextracted heated gluten, significant activity was obtained with strain TA98 with microsomal activation (Table I). At 5 MGE/plate or above with microsomal activation, aqueous acetonitrile extracts of heated gluten exhibited high activity in strain TA98 and weak activity with strains TA100 and TA102 (Table I). Extracts of unheated gluten were inactive (Table I).

Gluten also was baked in a vacuum oven in order to evaluate the effect of oxygen on browning. A 90% acetonitrile extract yielded 8% soluble product, much lower than for the product heated in air (24%). The revertant count of the vacuum-heated gluten extract with activation was high at 100 MGE/plate, but it decreased sharply with dosage. When calculated on the basis of extract yield weights, the response data are compatible with those obtained with the product baked in air (Table I). This result suggests that the same mutagen(s) may be formed in vacuum as in air, but in much smaller quantity.

When various carbohydrates were heated in air under the same conditions, significant mutagenicity was observed

Table II. Mutagenicity of Heated Carbohydrates in *S. typhimurium* Strain TA98[a]

	heated		unheated			heated		unheated	
MGE	+S9	−S9	+S9	−S9	MGE	+S9	−S9	+S9	−S9
	Hydrocellulose (Extract No. 1)					Starch Extract			
100	(184), 224**	(46), 42	62*	32	100	[236]**, 200**	[43]**, 45**		
50	114**	43			50	110**	37		
25	86**	37			10	(69)**, 68**	(40), 30		
10	[56], 52	[24], 27	(41)	(24)	1	(37)	(28)		
1	[46]	[28]	(42)	(23)	0.1	(46)	(29)		
0.1	[36]	[28]	(34)[c]	(28)[c]	0	42	21		
0	37	27	(39)	(27)	0	(39)	(27)		
0	(44)	(27)	44	27	0	[44]	[27]		
0	[39]	[27]			x (p)	1.72 (0.0001)	0.17 (0.042)		
x (p)	1.62 (0.0001)	0.19 (0.003)	0.23 (0.0002)	0.06 (0.11)					
	Hydrocellulose (Extract No. 2)					Maltose Extract			
10	(281)**, 290**	(41), 28			100	[84]**, 56**	[56]**, 34*		
1	77	34			50	43	41		
0.1	52	28			20	41	39		
0	(48)	(32)			10	(50)	(31)		
0	57	33			1	(34)	(26)		
x (p)	23.36 (0.0001)	0.27 (0.56)			0.1	(41)	(25)		
					0	45	24		
	Amylose Extract				0	(38)	(27)		
100	(213)**	(72)**			0	[40]	[29]		
10	61*	26			x (p)	0.28 (0.0005)	0.19 (0.010)		
1	35	20							
0.1	46	23				Cellulose Extract			
0	38	27			100	[173]**, 132**	[46], 34	40	25
0	(40)	(29)			50	90	31		
x (p)	1.73 (0.0001)	0.48 (0.0001)			20			34	23
					10	(59), 50	(30), 29		
	HPMC Extract				5			33	20
100	(122)**, 80**	(50), 32			1	(35)	(29)		
50	64	28			0.1	(30)	(32)		
10	[55], 41	[37], 23			0	42	21	34	21
1	[36]	[27]			0	(39)	(27)		
0.1	[44]	[24]			0	[44]	[27]		
0	42	21			x (p)	1.13 (0.0001)	0.12 (0.11)	0.07 (0.0005)	0.05 (0.020)
0	(40)	(29)							
0	[38]	[27]							
x (p)	0.60 (0.0001)	0.14 (0 .089)							

	heated		unheated			heated		unheated	
mg/plate[b]	+S9	−S9	+S9	−S9	mg/plate	+S9	−S9	+S9	−S9
	Cellulose					Glucose			
10	46[c]	33[c]			10	38[c]	31[c]		
1	42[c]	22[c]			1	54[c]	23[c]		
0.1	32[c]	23[c]			0.1	42[c]	26[c]		
0	39[c]	24[c]			0	39[c]	24[c]		
x (p)	0.91 (0.10)	1.01 (0.076)			x (p)	0.60 (0.45)	0.67 (0.40)		
	Hydrocellulose					Sucrose			
10	(56), 74[c]	(32), 21[c]			10	43[c]	29[c]		
5	(43)	(36)			1	44[c]	22[c]		
1	62[c]	20[c]			0.1	34[c]	12[c]		
0.1	46[c]	26[c]			0	39[c]	24[c]		
0	39[c]	24[c]			x (p)	0.48 (0.52)	1.02 (0.20)		
0	(50)	(34)							
x (p)	1.54 (0.082)	0.09 (0.92)							

[a] See footnotes to Table I. Data enclosed in parentheses and brackets are from separate experiments from values without parentheses or brackets. [b] Doses designated as mg/plate are unextracted baked samples.

in acetonitrile extracts of cellulose, amylose, HMPC, starch, hydrocellulose, and (carboxymethyl)cellulose (Tables II and III). At 10–100 mg equiv of extract/plate, revertant frequencies were up to 5 times background in TA98 with S9 activation and generally 2 times or less background in strains TA100 and TA102 with S9. Cellulose, amylose, HPMC, and starch were moderately active, while maltose and CMC had a low activity (Tables II and III). Activity in TA98 was generally dependent on S9 activation (Table VI). Heating increased yields after extraction with aqueous acetonitrile. Divergent results were obtained with the two extracts of baked hydrocellulose in strain TA98. Both extracts of heated hydrocellulose showed maximum activity in strain TA98, with weak mutagenicity in Strains TA100, TA102, and TA1537 with microsomal activation (Table III). An extract of untreated hydrocellulose was inactive.

Baked D-glucose, lactose, sucrose, and cellulose were also extracted with ethanol (Table VII). These extracts exhibited no mutagenicity with strain TA98 at doses of 20–100 MGE (results not shown).

In order to evaluate our results for the heated gluten

Table III. Mutagenic Activity of Heated Carbohydrates in *S. typhimurium* Strains TA100, TA102, TA1537, and TA2637[a]

	TA100				TA102				TA1537				TA2637	
	heated		unheated		heated		unheated		heated		unheated		heated	
	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
					Hydrocellulose Extract (Experiment No. 1, Extract 1)									
100	301**	230	180	187	687**	481	577	375	34*	19	17	12		
0	168	176	168	176	481	371	481	371	21	18	21	18		
					Hydrocellulose Extract (Experiment No. 2, Extract 2)									
10	242**	176			649	386								
0	144	151			543	382								
					Hydrocellulose (Milligrams per Plate)									
10	127	136			341	224							50	40
5	125	129			348	211							46	39
0	135	128			350	268							44	33
					Starch Extract									
100	288**	225			746**	488			45**	44**				
0	168	176			481	371			21	18				
					Maltose Extract									
100	378**	437**			747**	633**			19	7				
0	188	199			489	338			8	10				
					Cellulose Extract									
100	369**	389			872**	680**			37*	35*				
0	168	176			481	371			21	18				
					Amylose Extract									
100	312**	361**			685**	497*			29**	24*				
0	188	199			489	338			8	10				
					HPMC Extract									
100	264*	305**			708**	503*			27**	16				
0	188	199			489	338			8	10				

[a] See footnotes to Table I. All values in MGE with the exception of unextracted hydrocellulose (expressed as milligrams per plate). There were some heterogeneity problems for strains TA100 and TA102 that necessitated separate variance estimation for controls and treatment. The following LSD's can be used to compare treatment and control means [strain, 5% LSD, 1% LSD, 0.1% LSD, respectively]: TA100, 42.1, 58.4, 81.2; TA102, 97.6, 135.5, 188.9; TA1537, 9.38, 12.9, 17.5; TA2637, 15.6, 23.6, 38.0. The baked hydrocellulose was analyzed differently due to the three dose levels. There is weak evidence of an S9 × dose quadratic interaction for strain TA102. Otherwise, the main effect of S9 is significant for strains TA102 and TA2637, and neither dose nor S9 is significant for strain TA100.

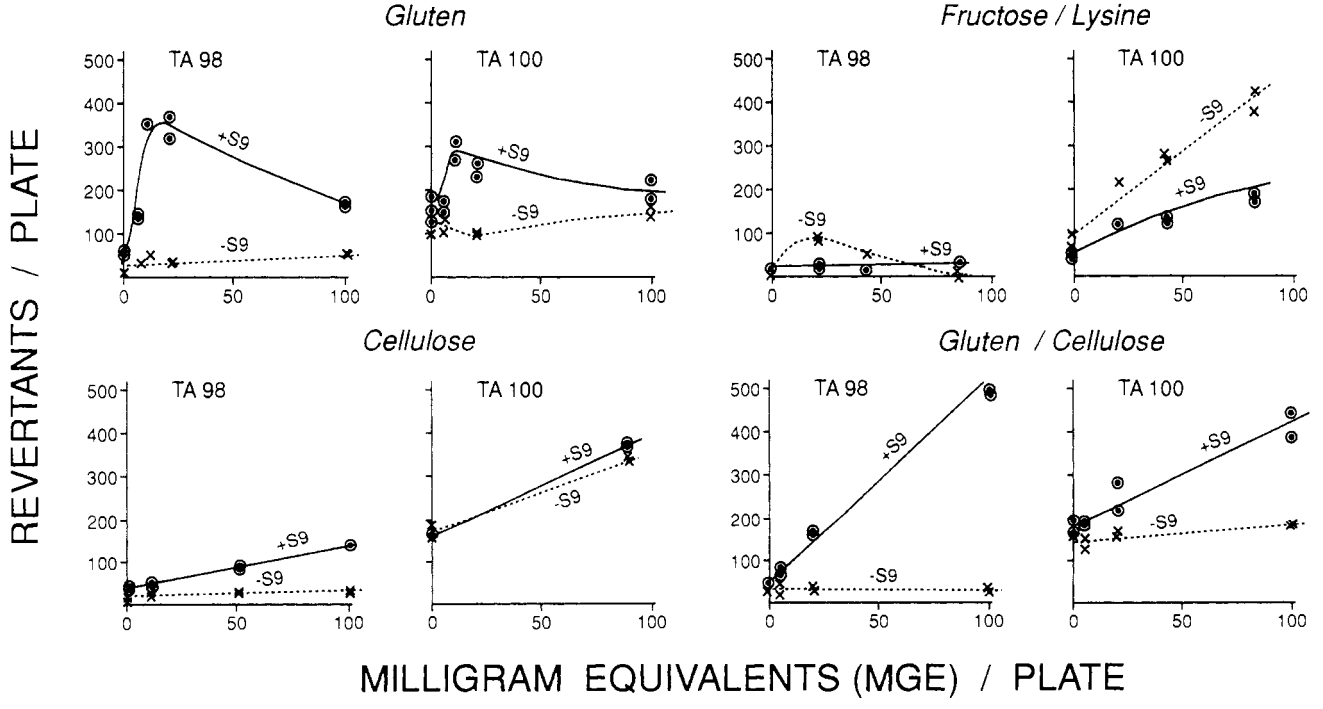

Figure 1. Mutagenicity of baked gluten, cellulose, and gluten/cellulose mixture and of heated aqueous fructose/lysine mixture in *S. typhimurium* strains TA98 and TA100.

and carbohydrate mixtures in relation to previous studies of browned Maillard reaction products (melanoidins), three different samples of an equimolar mixture of aqueous L-lysine and D-fructose were heated at 121 °C (Macgregor et al., 1989) and assayed with *S. typhimurium* strains. Figure 1 contrasts the mutagenicity of this mixture with that of dry-baked gluten, cellulose, or 80% gluten/20% cellulose. It can be seen that in the L-lysine/

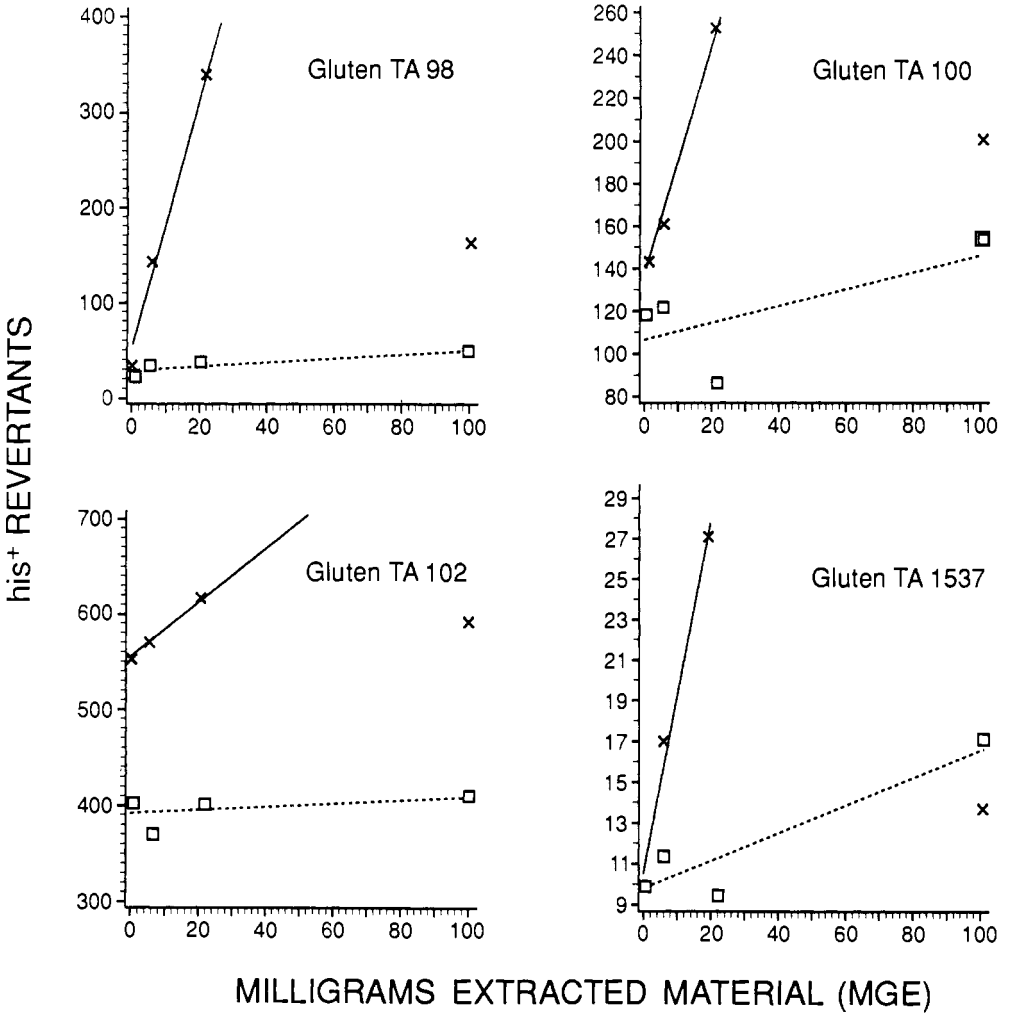

Figure 2. Mutagenicity of heated gluten extracts in four strains of *S. typhimurium*: (×) +S9; (□) −S9.

D-frutose mixture activity was greatest in strain TA100 and was decreased by S9, while in the gluten/cellulose samples activity was greater in strain TA98 than TA100 and was increased by S9.

Figures 2–4 show plots of the 12 reaction systems of this study. All compounds were tested up to the level of toxicity. Linear dose–response is demonstrated at lower doses in all cases.

Samples of gluten also were heated after blending with 20% of various food carbohydrates: reducing sugars, anhydrous β-D-glucose, maltose monohydrate, α-lactose monohydrate; nonreducing sugar, sucrose; starches, wheat starch, potato amylose; nonnutritive bulking fiber, cellulose, hydrocellulose; cellulosic additives, CMC and HPMC; vitamin C, sodium L-ascorbate and L-ascorbic acid. Most blends yielded mutagenic extracts, but activity did not seem to exceed levels that could be explained by the individual ingredients alone (cf. Tables IV and V with Tables I–III). Although these blend extracts exhibited levels of activity comparable to that of the baked whole gluten on a gram-equivalent (GE) basis, they were much more mutagenic on an absolute gravimetric basis, due to their small extract yields.

This was particularly striking with the baked gluten/cellulose blend extract (Table VI). It had the lowest yield (2%) but with strain TA98 was twice as active as any other blend extract at the GE dose levels tested (Table IV). The plate frequency of 150 revertants/5 MGE is equivalent to 1316 revertants/mg of extract; the corresponding activity for whole gluten (Table I) is 106–144 revertants/mg of extract.

DISCUSSION

Mutagenic Activities of Heated Foods. The mutagenic activity of commercial gluten heated without added carbohydrate may be due to thermal interaction with contaminant carbohydrate (11%) and/or lipid (8%), rather than from decomposition of the protein alone. Such a mechanism would be comparable with that occurring in the gluten/carbohydrate blends.

The difference in strain specificity and S9 effect in the heated L-lysine/D-fructose (MacGregor et al., 1989) suggests that the mutagens obtained are different and may have been formed by a different pathway. (Figure 1). Thus, the mutagenicity data parallel our previous findings on the chemical and antinutritional properties of these baked products (Friedman et al., 1987). The data also suggest that a Maillard-type chemical reaction between amine and reducing sugar does not play a significant role in the observed browning and/or mutagenesis (Taylor et al., 1986; Hargraves and Pariza, 1984).

Other evidence argues against involvement of amino–carbonyl reactions in the formation of heated gluten mutagens in that the mutagenicity of gluten blended with reducing sugars was comparable to that of gluten blended with nonreducing sugars or polysaccharides. Our observations on the behavior of the lysine/fructose system support related studies on mutagen formation during browning of amino acid/carbohydrate mixtures in aqueous solution (Spingarn et al., 1983; Shinohara et al., 1983; Cuzzoni et al., 1989). These authors report that (1) TA100 is the strain most sensitive to these browning products and (2)

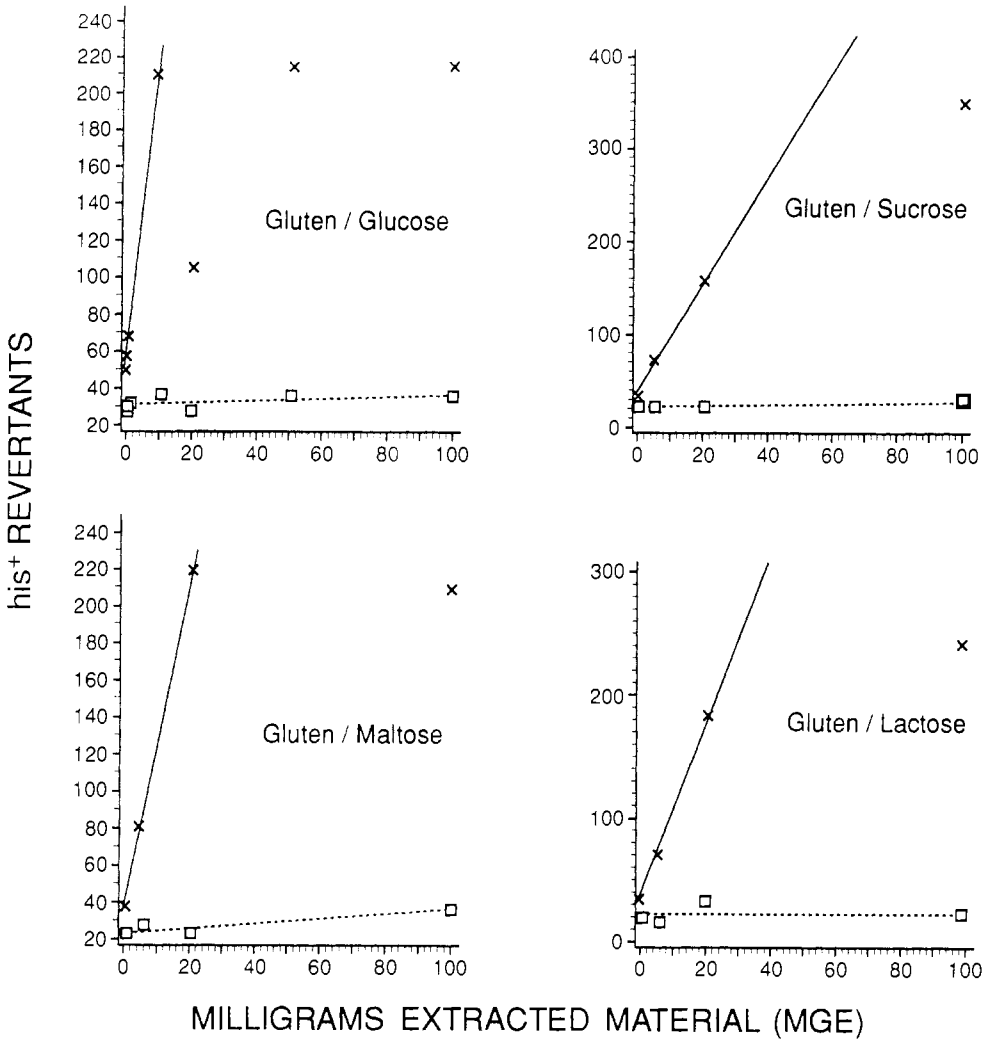

Figure 3. Mutagenicity of heated gluten/glucose, gluten/sucrose, gluten/maltose, and gluten/lactose extracts in *S. typhimurium* strain TA98: (×) +S9; (□) -S9.

with this strain there is a correlation among reaction temperature, extent of browning in the aqueous system, levels of furfurals formed during browning, and mutagenicity.

The evident lack of Maillard-type browning in crusty goods baked at about 200 °C does not imply its absence from foods (including baked crumb) that have been cooked, stored, or processed in aqueous or moist media at much lower temperatures. High-temperature non-Maillardian browning is essentially aerobic (oxidative) and occurs in the absence of moisture (Ziderman and Friedman, 1985). In contrast, classical amino–carbonyl browning is anaerobic and has an absolute requirement for water (Mauron, 1981; Taylor et al., 1986). The genotoxic consequences of there being two distinct systems of nonenzymatic browning should be adequately addressed, particularly in evaluating findings obtained under differing experimental or cooking condition. Frying, broiling, and roasting may be more analogous to baking than to aqueous cooking, considering the temperatures involved.

The widely different gravimetric extract yields (Tables VI and VII) from the various baked products may be due mainly to loss of product by volatilization during the vigorous exothermic reaction caused by heating. This may be particularly true of the gluten/cellulose and gluten/hydrocellulose blends, whose internal temperatures rose to 377 and 342 °C, respectively, in a 215 °C oven, with a concomitant extract weight loss of 50% (Ziderman and Friedman, 1985). The higher temperatures may also polymerize mutagenic materials, preventing their dissolution in the extraction.

Two factors might explain the behavior of gluten heated in a vacuum oven: (a) lack of oxygen; (b) a drying effect. This effect could result from the fact that heating samples in vacuum often subjects them to low vapor pressure and dries samples much more quickly than heating at atmospheric pressures.

A genotoxic selectivity for strains sensitive to frameshift rather than base-substitution mutations has been reported for cooked meats, e.g. by Felton et al. (1984) and Taylor et al. (1986). Taylor et al. also found an absolute requirement for metabolic activation. High levels of S9-dependent, frameshift-specific, mutagenic activity are displayed by organic extracts of amino acid/carbohydrate blends that have been dry-heated in air at 150–200 °C for 1–2 h and may or may not be related to our present results for gluten/carbohydrate blends. Most previous studies were carried out with strain TA98. To broaden the scope of the mutagenic response, we extended our studies to four additional strains listed in the tables (TA100, TA102, TA1537, TA2637). These strains differ in the molecular basis of the mutagenic response (Ames, 1989; Maron and Ames, 1983).

Significance for Food Safety. The formation of mutagens and carcinogens in foods during processing is a major area of concern for human health and safety. Human carcinogenic hazard associated with dietary mutagens and carcinogens has been estimated by an index called the

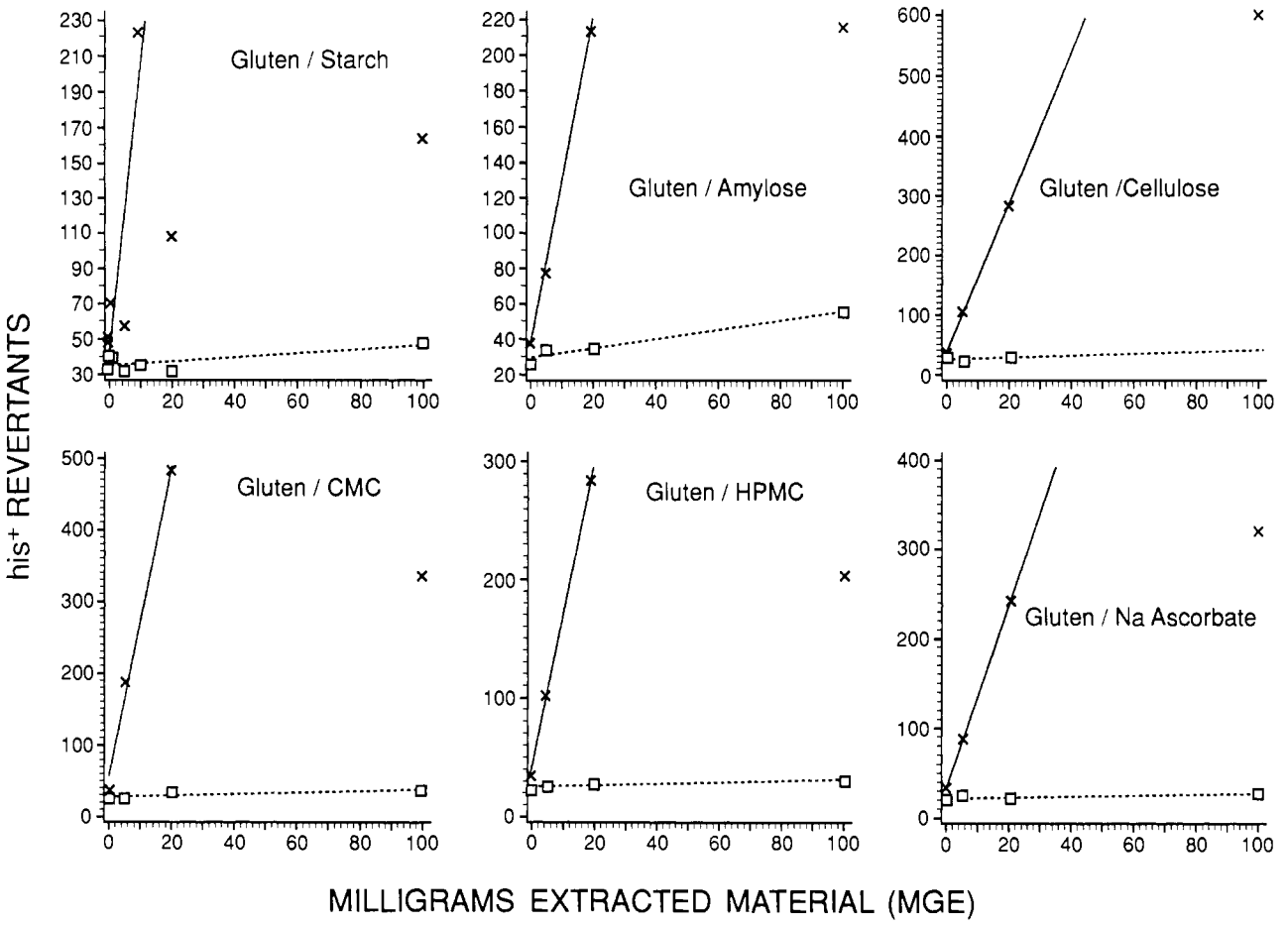

Figure 4. Mutagenicity of heated gluten/starch, gluten/amylose, gluten/cellulose, gluten/(carboxymethyl)cellulose (CMC), gluten/[(hydroxypropyl)methyl]cellulose (HPMC), and gluten/sodium ascorbate extracts in *S. typhimurium* strain TA98: (×) +S9; (□) -S9.

human exposure/rodent potency ratio (HERP) (Ames et al., 1987). This is the ratio of the chronic human exposure level to the lifetime dose rate in rodents that results in 50% of the animals being tumor-free, both human and animal dosages being expressed per unit of body weight. This method was applied to nine heterocyclic amines isolated from cooked foods and found to be potent carcinogens in rodents (Sugimura, 1986). Based on estimated daily human intake of 100 μg of heterocyclic amines, HERP was calculated at 0.02%, a value indicating these browning products to be a potentially significant dietary risk to man (Weisburger, 1987).

Bjeldanes et al. (1982) estimated the daily mutagen intake from cooked protein-rich foods in the average American diet, including beef, eggs, pork, ham, roast beef, chicken, milk, bread, and fish. The total mutagenic activity (assayed with *S. typhimurium* TA1538) ingested from cooked proteins in an average American diet is equivalent to 5600 revertants/day, more for people who consume their food well-done or overdone. These mutagens are formed during normal cooking, baking, and broiling as done by the average household. In a review on mutagen formation during commercial processing of foods, Krone et al. (1986) report that, in foods subjected to commercial baking, canning, dehydration, and related thermal treatments, levels of bacterial mutations are 3–17 times greater than spontaneous rates. Mutagen formation was related to heating time and to processing temperature, which ranged from about 100 to 200 °C. This survey suggests that mutagen formation during commercial food processing is largely associated with formation of browning products and that perhaps mutagens produced in different foods, e.g., bread, fish, fruits, and meat, may not be chemically identical.

To place our findings in perspective, it should be noted that the potential intake of mutagens and carcinogens from food sources is high relative to mutagens known to be a significant human risk. For example, the estimated daily intake of aflatoxins in the U.S. diet (0.017 μg) and in areas of Thailand in which human liver damage and cancer possibly due to aflatoxin were reported (3.7 μg) (Carlborg, 1979) are equivalent to 68 and 15 000 revertants/day, respectively. The mutagenic activity in the smoke of one cigarette ranges from about 1000 to 10 000 revertants, depending on tar content (Kier et al., 1974). It is also worth noting that heating does not seem to significantly increase the mutagenicity of fruit juices (Friedman et al., 1990).

Our findings and these considerations lead to the following future objectives: First, it may be possible to modify food-processing conditions to minimize mutagens, in view of the observed inhibition of nonenzymatic browning and aflatoxin mutagenicity by SH-containing amino acids such as cysteine, *N*-acetyl-L-cysteine, and reduced glutathione (Molnar-Perl and Friedman, 1989; Friedman et al., 1982). (Additional studies are needed to show whether inhibition of browning is parallled by decrease in mutagen formation.) Second, browning reactions produce, in addition to mutagens, antioxidants and antimutagens (Wang et al., 1982; Powrie et al., 1986). Conditions need to be devised to favor formation of the beneficial compounds at the expense of mutagens. Third, we recently discovered that the in vitro genotoxicity of Maillard amino acid/carbohydrate browning products does

Table IV. Mutagenicity of Heated 80% Gluten/20% Carbohydrate Mixtures in *S. typhimurium* Strain TA98

MGE	heated +S9	heated −S9
	Gluten/Maltose Extract	
100	207**	36
20	221**	24
5	82	27
0	37	22
x (p)	9.20 (0.0001)	0.12 (0.010)
	Gluten/Sodium Ascorbate Extract	
100	319**	27
20	241**	23
5	89**	25
0	34	21
x (p)	10.29 (0.0001)	0.05 (0.44)
	Gluten/Lactose Extract	
100	228**	22
20	176**	30
5	68	17
0	34	21
x (p)	7.12 (0.0001)	0.01 (0.87)
	Gluten/CMC Extract	
100	(427)**, 246**	(36), 36
20	(453)**, 514**	(37), 30
5	189*	27
0	(40)	(33)
0	37	22
x (p)	21.85 (0.0001)	0.09 (0.57)
	Gluten/Sucrose Extract	
100	325**	26
20	149**	20
5	69**	20
0	34	21
x (p)	5.68 (0.0001)	0.06 (0.21)
	Gluten/Hydrocellulose Extract	
10	(416)**, 337**	(56), 42
1	73	34
0.1	52	44
0	(68)	(46)
0	48	35

MGE	heated +S9	heated −S9
	Gluten/Amylose Extract	
100	204**	53*
20	203**	36
5	76**	35
0	37	22
x (p)	8.24 (0.0001)	8.24 (0.0001)
	Gluten/HPMC Extract	
100	(240)**, 164**	(33), 30
20	(269)**, 297**	(35), 27
5	104*	29
0	(40)	(33)
0	37	22
x (p)	12.17 (0.0001)	0.03 (0.72)
	Gluten/Ascorbic Acid Extract	
10	[(197), 201]*	(32), 36
1	66	34
0.1	50	36
0	57	33
0	(48)	(32)
x (p)	14.83 (0.0001)	−0.04 (0.87)
	Gluten/Glucose Extract	
100	222**	33
50	213**	33
20	105**	28
10	[217]**, (200)**	(37), [37]
1	[68]*	[32]
0.1	[58]	[31]
0	45	[33]
0	(48)	(32)
0	[57]	24
x (p)	15.70 (0.0001)	0.02 (0.78)
	Gluten/Starch Extract	
100	162**	48
20	108**	32
10	[196]**, (246)**	[37], (34)
5	58	32
1	(71)	(40)
0.1	(52)	(41)
0	40	33
0	(57)	(33)
0	[48]	[32]
x (p)	16.19 (0.0001)	0.12 (0.46)

Gluten/Cellulose Extract

MGE	heated +S9	heated −S9	unheated +S9	unheated −S9
100	(490)**, 694**	(35), 32	74**	32
20	(167)**, 397*	(35), 24	65*	36
5	(66), 151	(32), 20	46	35
0	34	21		
0	(40)	(41)	40	41
x (p)	12.10 (0.0001)	0.05 (0.88)	1.25 (0.0005)	−0.06 (0.22)

mg/plate	heated +S9	heated −S9
	Gluten/Hydrocellulose	
1.81	69*	46
0.181	36	44
0.0181	49	35
0	48	35

[a] See footnotes to Table I. The +S9 treatment appears to have resulted in multiphasic responses. Since there are insufficient design points to fit these more complex models, one or more of the high doses for this treatment were usually deleted for the regression analyses, which were then all linear. For most samples, only the 100-MGE dose was deleted. Samples that required no deletions are gluten/ascorbic acid an d gluten/hydrocellulose. The gluten/glucose and gluten/starch extracts required deletions of doses of 20 MGE for the +S9 treatment.

Table V. Mutagenicity of Heated 80% Gluten/20% Carbohydrate Mixtures in *S. typhimurium* Strains TA100, TA102, and TA1537

	TA100				TA102				TA1537			
	heated		unheated		heated		unheated		heated		unheated	
MGE	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
					Gluten/Glucose Extract							
10	230**	143			666*	379			7	3		
0	144	151			543	382			5[c]	1[c]		
					Gluten/Ascorbic Acid Extract							
10	228**	151			611	441			8	4		
0	144	151			543	382			5[c]	1[c]		
					Gluten/Cellulose Extract							
100	419**	185	167	168	760	439	714	458	39**	12	14	8
20	250**	165	199	177	732	375	725	423	22*	10	12	14
5	186	139	177	168	686	418	740	405	15	13	13	11
0	174	146	174	146	647	383	647	383	11	13	11	13
x (p)	2.41 (0.0001)	0.40 (0.073)	0.13 (0.54)	0.09 (0.67)	0.89 (0.076)	0.46 (0.28)	0.20 (0.68)	0.64 (0.23)	0.26 (0.0001)	0.00 (0.92)	0.02 (0.34)	0.04 (0.094)
					Gluten/Hydrocellulose Extract							
10	327**	207			721**	444			12	5		
0	171	168			559	417			5[c]	1[c]		
					Gluten/Starch Extract							
10	224**	173			692	414			12	6		
0	144	151			543	382			5[c]	1[c]		

[a] See footnotes to Table I. Analyses of variance were only run for the gluten/cellulose extract data. Comparisons between treated and control means for the other samples can be based on the following LSD's computed from pooled variance estimates within strains [strain, 5% LSD, 1% LSD, 0.1% LSD, respectively]: TA100, 42.2, 57.0, 76.0; TA102, 76.8, 103.8, 138.5; TA1537, 9.94, 10.7, 14.3.

Table VI. Extraction of Foodstuffs with Aqueous Acetonitrile[a]

	weight extract/g			
	alone		gluten blend	
carbohydrate	untreated	heated	untreated	heated
hydrocellulose	0.023	0.120	nd	1.1[b]
cellulose	0.022	0.189	0.338	0.46
HPMC	0.496	2.81	0.410	3.55
CMC	0.031	0.249	0.348	3.55
starch	0.015	0.171	nd	2.56[b]
amylose	0.011	0.586	0.271	2.89
maltose	0.301	1.74	0.574	1.48
sodium L-ascorbate	8.62	0.459	0.546	3.85
L-ascorbic acid	19.4	3.12	nd	2.46[b]
lactose	nd	nd	0.328	1.90
sucrose	nd	nd	0.618	0.80
D-glucose	nd	nd	nd	1.26[b]
gluten	0.37	4.8[b]		
vacuum-baked gluten	nd	1.62		

[a] 20-g samples extracted with acetonitrile/water (9:1), except where noted otherwise. [b] Extracted with acetonitrile/water (10:1). With acetonitrile/water (10:1), the extract weight of heated hydrocellulose was 0.53 g. nd = not determined.

Table VII. Extraction of Heated Carbohydrates with Ethanol

baked carbohydrate[a]	extr/mg	baked carbohydrate[a]	extr/mg
D-glucose	34	sucrose	828
lactose	54	cellulose	13

[a] 20-g samples.

not correlate with in vivo clastogenicity (chromosome-damaging effect) in mouse bone marrow cells (MacGregor et al., 1989). These results suggest that browning products may not be sufficiently absorbed into the general vascular circulation or that active compounds may be metabolized to inactive forms. This raises broad questions regarding the prognostic value of bacterial mutagenicity for food safety. Last, in spite of the cited considerations, and the current debate about the relative importance of naturally occuring versus processing induced food toxicants (Ames et al., 1987), our results suggest that conditions need to be developed to minimize or prevent the formation of mutagenic compounds during food processing (De Flora, 1989; Molnar-Perl and Friedman, 1989; Friedman, 1984; Stich and Rosin, 1984).

ACKNOWLEDGMENT

We thank Carol E. Levin and Melody Hung for the heating experiments and for preparing the extracts and Bruce E. Mackey for the statistical analyses. Hung's work was done as part of a course, Nutritional Field Studies, sponsored by the Department of Nutritional Sciences, University of California, Berkeley. We are grateful to Dr. J. T. MacGregor for helpful advice.

LITERATURE CITED

Ames, B. N. Mutagenesis and carcinogenesis: endogenous and exogenous factors. *Environ. Mol. Mutagen.* **1989**, *14*, 66–77.

Ames, B. N.; Magaw, R.; Gold, I. S. Ranging possible carcinogenic hazards. *Science* **1987**, *236*, 271–280.

Bjeldanes, L. F.; Morris, M. M.; Felton, J. S.; Healy, S.; Stuermer, D.; Berry, P.; Timourian, H.; Hatch, F. T. 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.* **1982**, *20*, 357–363.

Bjeldanes, L. F.; Felton, J. S.; Hatch, F. T. Mutagens in cooked food: chemical aspects. In *Nutritional and Toxicological Aspects of Food Safety*; Friedman, M., Ed.; Plenum: New York, 1984; pp 545–554.

Carlborg, F. W. Cancer, mathematical models, and aflatoxin. *Food Cosmet. Toxicol.* **1979**, *17*, 159–166.

Cuzzoni, M. T.; Stoppini, G.; Gazzani, G. Effect of storage on mutagenicity, absorbance and content of furfural in the ribose-lysine Maillard system. *Food Chem. Toxicol.* **1989**, *27*, 209–213.

De Flora, S. Role of glutathione and N-acetylcysteine as inhibitors of mutagens and carcinogens. In *Absorption and Utilization of Amino Acids*; Friedman, M., Ed.; CRC Press: Boca Raton, FL, 1989; Vol. 3, Chapter 3.

Felton, J. S.; Bjeldanes, L. F.; Hatch, F. T. Mutagens in cooked foods – metabolism and genetic toxicity. In *Nutritional and Toxicological Aspects of Food Safety*; Friedman, M., Ed.; Plenum: New York, 1984; pp 555–566.

Friedman, M. Chemically reactive and unreactive lysine as an index of browning. *Diabetes* **1982**, *31*, Supplement 3, 5–14.

Friedman, M. Sulfhydryl groups and food safety. *Adv. Exp. Med. Biol.* **1984**, *177*, 31–64.Friedman, M.; Cuq, J. L. Chemistry, analysis, nutritional value, and toxicology of tryptophan in food. A review. *J. Agric. Food Chem.* **1988**, *36*, 1079–1093.

Friedman, M.; Wehr, C. M.; Schade, J. E.; MacGregror, J. T. Inactivation of aflatoxin B_1 mutagenicity by thiols. *Food Chem. Toxicol.* **1982**, *20*, 887–892.

Friedman, M.; Gumbmann, M. R.; Ziderman, I. I. Nutritional value and safety in mice of proteins and their admixtures with carbohydrates and vitamin C after heating. *J. Nutr.* **1987**, *117*, 508–518.

Friedman, M.; Wilson, R. E.; Ziderman, I. I. Effect of heating on mutagenicity of fruit juices in the Ames test. *J. Agric. Food Chem.* **1990**, *38*, 740–743.

Fujimaki, M., Namiki, M., Kato, H., Eds. *Amino-Carbonyl Reactions in Food and Biological Systems*; Elsevier: Amsterdam, Tokyo, 1986.

Gazzini, G.; Vagnarelli, P.; Cuzzoni, M. T.; Mazza, P. G. Mutagenic activity of the Maillard reaction products of ribose with different amino acids. *J. Food Sci.* **1987**, *52*, 757–760.

Hargraves, W. A.; Pariza, M. W. Mutagens in cooked foods. *J. Environ. Sci. Health* **1984**, *C2*, 1–49.

Hatch, F. T.; Knize, M. G.; Healy, S. K.; Slezak, T.; Felton, J. S. Cooked-food mutagen reference list and index. *Environ. Mol. Mutagen.* **1988**, *12*, 1–85.

Hayashi, T.; Shibamoto, T. Analysis of methyl glyoxal in foods and beverages. *J. Agric. Food Chem.* **1984**, *33*, 1090–1093.

Kier, L. D.; Yamasaki, E.; Ames, B. N. Detection of mutagenic activity in cigarette smoke condensate. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, *71*, 4159–4163.

Krone, C. A.; Yeh, S. M. J.; Iwaka, W. T. Mutagen formation during commercial processing of foods. *Environ. Health Perspect.* **1986**, *67*, 75–88.

Leszkowiat, M. J.; Barichello, V.; Yada, R. Y.; Coffin, R. H.; Lougheed, E. C.; Stanley, D. W. Contribution of sucrose to nonenzymatic browning in potato chips. *J. Food Sci.* **1990**, *55*, 281–283.

MacGregor, J. T.; Tucker, J. D.; Ziderman, I. I.; Wehr, C. M.; Wilson, R. E.; Friedman, M. Nonclastogenicity in mouse bone marrow of fructose/lysine and other sugar/amino acid browning products with *in vitro* genotoxicity. *Food Chem. Toxicol.* **1989**, *27*, 715–721.

Maron, D. M.; Ames, B. N. Revised methods for *Salmonella* mutagenicity test. *Mutat. Res.* **1983**, *113*, 173–215.

Mauron, J. The Maillard reaction in food; a critical review from the nutritional standpoint. *Prog. Food. Nutr. Sci.* **1981**, *5*, 5–35.

Miller, A. J. Thermally induced mutagens in protein foods. In *Protein Quality and Effects of Processing*; Phillips, R. D., Finley, J. W., Eds.; Marcel Dekker: New York, 1988; pp 145–188.

Molnar-Perl, I.; Friedman, M. Inhibition of browning by sulfur amino acids. *J. Agric. Food Chem.* **1989** (submitted for publication).

Pariza, M. W.; Ashoor, S. H.; Chu, F. S. Mutagens in heat-processed meat, baked and cereal products. *Food Cosmet. Toxicol.* **1979**, *17*, 429–430.

Powrie, W. D.; Wu, C. H.; Molund, V. P. Browning reaction systems as sources of mutagens and antimutagens. *Environ. Health Perspect.* **1986**, *67*, 47–54.

SAS Institute Inc. *SAS/STAT Guide for Personal Computers*, Version 6; SAS Institute: Cary, NC, 1987.

Satterthwaite, F. E. An approximate distribution of estimates of variance components. *Biom. Bull.* **1946**, *2*, 110–114.

Shibamoto, T. Occurrence of mutagenic products in browning model systems. *Food Technol.* **1982**, 59–62.

Shinohara, K.; Yahan, N.; Tanaka, M.; Yamamoto, K.; Wu, R. T.; Murakami, H.; Omura, H. Formation of mutagens by aminocarbonyl reactions. *Mutat. Res.* **1983**, *122*, 279.

Spingarn, N. E.; Garvie-Gould, C. T.; Slocum, L. A. Formation of mutagens in sugar-amino acid model systems. *J. Agric. Food Chem.* **1983**, *31*, 301–304.

Stich, H. F.; Rosin, M. P. Naturally occurring phenolics as antimutagenic and anticarcinogenic agents. In *Nutritional and Toxicological Aspects of Food Safety*; Friedman, M., Ed.; Plenum: New York, 1984; pp 1–30.

Stich, H. F.; Rosin, M. P. Towards a more comprehensive evaluation of a genotoxic hazard in man. *Mutat. Res.* **1985**, *150*, 43–50.

Sugimura, T.; Sato, S.; Ohgaki, M.; Takayama, S.; Nagao, N.; Wakabayeshie, K. Mugtagens and carcinogens in cooked food. In *Genetic Toxicology of the Diet*; Knudsen, I. B., Ed.; Alan R. Liss: New York, 1986; pp 85–107.

Taylor, R. T.; Fultz, E.; Knize, M. Mutagen formation in model beef supernatant fraction. IV. Properties of the system. *Environ. Health Perspect* **1986**, *67*, 59–74.

Van der Hoeven, J. C. M. Mutagens in food products of plant origin. In *Mutagens in Our Environment*; Knudsen, I. B., Ed.; Alan R. Liss: New York, 1982; pp 327–338.

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

Weisburger, J. H. On the mechanism relevant to nutritional carcinogenesis. *Prev. Med.* **1987**, *16*, 586–591.

Ziderman, I. I.; Friedman, M. Thermal and compositional changes of dry wheat gluten-carbohydrate mixtures during simulated crust baking. *J. Agric. Food Chem.* **1985**, *33*, 1096–1102.

Ziderman, I. I.; Gregorski, K. S.; Lopez, S. V.; Friedman, M. Thermal interaction of ascorbic acid and sodium ascorbate with proteins in relation to nonenzymatic browning and Maillard reactions in foods. *J. Agric. Food Chem.* **1989**, *37*, 1480–1486.

Received for review August 28, 1989. Accepted January 8, 1990.

Registry No. HPMC, 9004-65-3; CMC, 9004-32-4; amylose, 9005-82-7; starch, 9005-25-8; cellulose, 9004-34-6; maltose, 69-79-4; sucrose, 57-50-1; glucose, 50-99-7; lactose, 63-42-3; sodium ascorbate, 134-03-2; ascorbic acid, 50-81-7.