Browning Reaction Systems as Sources of Mutagens and Antimutagens

by William D. Powrie,* Chiu H. Wu,* and V. Paul Molund*

Heated food systems contain hundreds of chemical compounds, some being mutagenic and others being antimutagenic. Studies have indicated that foods exposed to drying, frying, roasting, baking, and broiling conditions possess net mutagenic activity as assessed by the Ames/Salmonella/microsomal mutagenicity test and the chromosome aberration assay with Chinese hamster ovary (CHO) cells. With the above-mentioned heat treatment of food, nonenzymic browning reactions are generally proceeding at rapid rates and are involved in the development of mutagens.

Caramelization and Maillard reactions are two important pathways in the nonenzymic browning of food and are responsible for the formation of volatile aromatic compounds, intermediate nonvolatile compounds, and brown pigments called melanoids.

Heated sugar–amino acid mixtures possessed mutagenic activities which have been assessed by short-term bioassays. Purified Maillard and caramelization reaction products such as reductones, dicarbonyls, pyrazines, and furan derivatives have exhibited mutagenicity and clastogenicity.

The water-insoluble fraction (WIF) of instant coffee and a model-system melanoidin (MSM) have been shown to inhibit the mutagenicity of known carcinogens— aflatoxin B₁ (AFB₁), N-methyl-N′-nitro-N′-nitrosoguanidine (MNNG), and benzo(a)pyrene (BP)—in aqueous dispersion. WIF and MSM were found to be effective binding agents for the carcinogens.

Introduction

Heat treatment of food is used extensively to increase the palatability of food. Baking, roasting, broiling, and frying of food bring about the formation of flavor compounds and brown pigments. Flavor compounds, including volatile odoriferous substances and nonvolatile bitter material, are formed by degradation of sugars and amino acids. With some food products, brown pigments produced during heating are desirable visual attributes which convey flavor expectation.

Baked and fried flavors along with brown pigment formation in food are formed by thermal degradation of sugars and amino acids at sites where extensive evaporative water loss has occurred during heating. Such heat treatment of foods includes exposure to hot oil (frying), heating by hot air (baking and roasting), and direct exposure to radiant energy sources such as a hot electrical element or glowing coals (broiling). Infrared radiation from red-hot elements and glowing coals is a major source of heat energy in the browning process. With metal pan and air heating, as well as infrared heating of food, a temperature gradient develops in the food since heat is transferred inwardly from the surface. Furthermore, mass transfer of water (as vapor and sometimes as drip) progresses during baking, broiling and frying of food, and a moisture gradient is established with the highest moisture content in the center of the food product (1, 2).

The rapid evaporation of water in the surface region of meat patties leads to the creation of a crust with a low free water content. Melted fat from meat can readily absorb heat energy from the metal pan to bring the crust temperature of meat above 100°C (3). The combination of high temperature, absence of free water, and high concentration of reactants is responsible for the rapid browning of the crust through the Maillard reactions. Maillard reactions do not occur normally in the interior of meat patties because of the relatively low temperature and high moisture content (4).

Pyrolysis is a term used to denote the process of thermal degradation of constituents in food heated to temperatures much higher than 100°C in the absence of free water. During the roasting of coffee and cocoa beans, amino acids and sugars are decomposed at temperatures up to about 220°C. Pyrolytic reactions can occur in the crust of baked bread and fried steak when free water is not present. Nonenzymic browning reaction pathways are involved in the pyrolysis treatment of food systems.

Nonenzymic Browning Reactions

The two major types of nonenzymic browning reactions are Maillard or carbonyl-amine reactions and car-
amalization. Maillard reactions in aqueous and dehydrated food systems involve the decomposition of reducing sugars in the presence of free amino acids, peptides, and polypeptides. In the pathway of carmelization only reducing sugars participate in the reactions. Presumably carmelization as well as Maillard reactions occur in heated protein foods. Several comprehensive reviews on nonenzymic browning have been published recently (5, 7).

Both Maillard reactions and carmelization involve ring-opening and enolization of reducing sugars. With a rise in pH of sugar solutions, conversion of the cyclic hemiacetal forms to acyclic (open-chain) carbonyls is enhanced (9). The amount of carbonyl form in a sugar solution is relatively small. For example, only 0.02% aldehyde form is present in a d-glucose solution at about 25°C, and in a d-xylene solution, about 0.17% of the aldehyde form exists. The highest percentage of aldehyde form has been reported to be in a d-ribose solution. Pentoses bring about browning at a greater rate than hexoses because of the differences in concentrations of the aldehyde form and ring-opening rates (9).

Chemical events of carmelization involve enolization of open-chain reducing sugars, dehydration, fragmentation, and polymerization of reaction products to form brown pigments (10). In neutral and weakly alkaline media, enolization of reducing sugars is rapid and dehydration is slow, whereas in acidic media, enolization is slow and dehydration is rapid. Carmelization proceeds a rapid rate when sugar-containing food systems in the hydrated and anhydrous forms are heated to 100°C and above. Enolization of reducing sugars is increased in the presence of organic acid or base catalysts to bring about the formation of enediols. For aldoses, 1,2-enediols are the dominant species. Sugar dehydration reactions consist of a sequence of chemical events for the elimination of water from the sugar molecules and the formation of intermediates called 3-deoxyxosones. The b-elimination scheme for sugar dehydration is shown in Figure 1. When acid-containing hexose solutions with low pH values are heated, 5-(hydroxymethyl)-2-furfural is formed by the elimination of three water molecules in the dehydration reactions. Low con-

![Figure 1](image1.png)

**Figure 1.** Formation of 3-deoxyxosone (10).

**Table 1. Characteristics of Maillard-type browning reactions.**

| Stage          | Color                          | Reactions                                                                 |
|----------------|--------------------------------|---------------------------------------------------------------------------|
| Initial        | Colorless; no absorption in    | Condensation                                                              |
|                | near-ultraviolet               | Enolization                                                               |
|                |                                | Amadori rearrangement                                                     |
| Intermediate   | Buff yellow; strong absorption | Sugar dehydration to 3-deoxyglucose and its -3,4-ene, HMF, and 2-          |
|                | in near-ultraviolet range      | (hydroxyacetyl)furan                                                      |
|                |                                | Sugar fragmentation                                                       |
|                |                                | Formation of a-dicarbonyl compounds, reductones, pigments                |
| Final          | Red-brown and dark brown color | Aldol condensations                                                       |
|                |                                | Polymerization                                                            |
|                |                                | Strecker degradation of α-amino acids to aldehydes and N-heterocyclics at |
|                |                                | elevated temperatures; carbon dioxide evolves                             |

centrations of other furans have been found in the low pH solutions. At pH levels of 7 and above, volatile aldehydes, ketones, and acids are formed through the fragmentation of dehydrated molecules. Acetol, acetal, diacetyl, acetic acid, and formic acid are a few of the more important fragmentation products (10).

In the advanced stages of carmelization, brown pigments are formed. They are formed by self- and cross-condensations of aldehydes and ketones which occur as fragmentation products.

Table 1 shows the reactions which occur during the three major stages in the Maillard-type browning reaction (10). In the first step of the Maillard reactions, an amino acid condenses with a reducing sugar to form a Schiff’s base (imine), which undergoes cyclization to N-substituted glycosylamine, as shown in Figure 2. Subsequently, the glycosylamine is converted to 1,2-eneiminol which is transformed to N-substituted 1-amino-1-deoxy-2-ketone by the Amadori rearrangement. This rearrangement involves the transformation of an aldose derivative to the ketone form. Amadori ketone compounds have been found in several foods. The reaction of ketones such as fructose with an amino acid can lead to the formation of ketosylamines which are converted to 2-amino-2-deoxyaldoses.

![Figure 2](image2.png)

**Figure 2.** Formation of N-substituted glycosylamine.
A major pathway of the Maillard reactions leads from the 1,2-eneaminol form of the Amadori compound to a 3-deoxyhexosone intermediate (Fig. 3). 2-Furfurals can be formed by the dehydration of 3-deoxyhexosone. A minor pathway of the Maillard reactions is the conversion of the 2,3-enediol to the methyl-α-dicarbonyl intermediate, which undergoes transformation to aldehydes, ketones, furans, pyrroles, quinolines, and indoles. Several cyclic flavor compounds, such as maltol and isomaltol, are formed from 2,3-enediol.

Another important pathway of the Maillard reactions is the Strecker degradation of amino acids (Fig. 4). When α-dicarbonyl compounds are heated with amino acids, nitrogen-containing carboxylic aldehydes are created and subsequently altered to form aldehydes, carboxylic acid, and pyrazines from the transamination reaction (10). The carbon dioxide released during the Maillard reactions originates from carboxyl groups of amino acids.

Brown pigments formed during the latter stages of the Maillard reactions have a wide variety of molecular weights. Motai (11) found that the molecular weights of melanoidins in a glycine-xylose solution (heated at 100°C for 2 hr) ranged from 290 to 14,200. The intensity of the brown color of melanoidin increased with the degree of polymerization. According to Benzing-Purdie and Ripmaster (12), the melanoidin fraction (molecular weights above 12,000) isolated from heated glycine-xylose solution had an elemental composition of 58.51% C, 6.78% H, and 4.33% N. Similar values were obtained for melanoidin fraction from a heated glycine-glucose solution. The melanoidins are considered to be aliphatic compounds with a large number of double bonds (13,14).

The melanoidin fractions, prepared by acid precipitation of polymers in a heated glycine-glucose solution, contained molecules with isoelectric points near 2.5 and with net negative charges at pH levels of 2.5 and above (15). Presumably, carboxyl groups were responsible for the negative charges on the melanoidins. When Horikoshi and Gomyo (16) mixed a high molecular weight melanoidin fraction with ovalbumin, a protein-melanoidin complex precipitated at pH values between the isoelectric points of melanoidin (pH 2.5) and ovalbumin (pH 4.6).

The factors influencing Maillard reactions in foods are duration and temperature of heating, pH, types of sugars and moisture content. According to Hurrell and Carpenter (17), almost as many ε-amino groups of lysine reacted in a albumin-glucose mixture after 30 days at 37°C as had reacted in the mixture heated at 121°C for 15 min. An intense brown color and flavor compounds in foods are brought about at temperatures of about 90°C and higher. Many studies have demonstrated that a rise in pH increases the rate of browning (18,19). Since acids are formed during Maillard reactions, buffers such as inorganic salts and proteins in foods will inhibit pH change during heat processing and thus the rate of
browning will not be altered appreciably. Mono- and\disaccharides with reducing groups can participate in\Maillard reactions. Lewis and Lea (20) reported that\the order of reactivity of sugars was xylose > arabinose >\glucose > lactose > maltose > fructose. As the mois-\ture content of a food is reduced, the rate of browning\increases because the reactant concentrations rise. The\optimum moisture content for Maillard browning de-\pends on the types of reactants and the storage tem-\perature. Lea and Hannan (21) found that a moisture\content of 15 to 18% brought about a maximum loss of\e-amino groups in a casein–glucose mixture stored at\37°C, but the maximum level of browning occurred at\a moisture level of 30%.

**Mutagenic Activity of Nonenzymic\Browning Reaction Products in\Model Systems**

Nonenzymic browning reactions are undoubtedly in-
volved in the chemical events leading towards mutagen\development in thermally treated foods. The assess-
ment of the mutagenic activity of heated sugar and\sugar-amino acid model systems should be helpful in\the elucidation of the role of browning reactions in mutagen\formation in heated complex food products.

Spingarn and Garvie (22) found that all methylene\chloride extracts of heated reducing sugar–NH₄OH\aqueous solutions possessed mutagenic activity when\assayed by the Ames/Salmonella/microsome test using\strain TA 98 after S9 activation. Preliminary studies\with refluxed rhamnose–NH₄OH solution revealed that\*S. typhimurium* TA 98 was more responsive than TA\100 strain, and that both strains were more responsive\when S9 was added to the solution. The sugars included\arabinose, 2-deoxyglucose, galactose, glucose, rham-
noise, and xylose. Studies with refluxed glucose–\NH₄OH solution showed a parallelism between muta-
gen formation and pyrazine development over an 80 min\time period. These investigators hypothesized that the\reactions that form pyrazines also form the mutagens.\However, it should be noted that pyrazines possessed\no mutagenic activity. When a glucose–NH₄OH system\was heated, propyl gallate acted as an inhibitor for mu-
tagen formation, whereas tryptophan enhanced the form-
ation.

After a Maillard browning reaction solution of L-
rhamnose, NH₃, and H₂S was extracted with dichoro-
methane, Toda et al. (23) noted a brown oily material\upon solvent removal. The oily material possessed mu-
tagenic activity with TA 98 and TA 100 after S9 acti-
vation. The solvent-extracted aqueous phase did not\display mutagenicity.

Commercial caramel is manufactured by heating a\sugar–NH₄OH solution at 120–160°C. Maillard reaction\products are present in the final colorant mixture. Stich\et al. (24) subjected CHO cells, *Saccharomyces cere-
visiae* strain D7, and Ames Salmonella strains TA 98\and TA 100, with and without S9 mix, to a solution of\a commercial caramel colorant. Mutagenic activity was\detected with both tester strains when caramel was

![Figure 5.](image)

*Figure 5.* Chromosome aberration of Chinese hamster ovary (CHO) cells treated with heated glucose-amino acid solutions (25): (●) initial pH 7; (▲) initial pH 10.
assessed with the Ames mutagenicity test. In addition, the caramel colorant was a potent inducer of gene conversion in the D7 yeast strain. Of particular interest was the observation that caramel caused a high frequency of chromosome aberrations in CHO cells.

Powrie et al. (25) employed three short-term tests to assess the clastogenic and point mutagenic activities of Maillard reaction sugar–amino acid solutions at initial pH values of 7 and 10. The amino acids used were arginine, lysine, histidine, serine, glutamine, and glutamic acid, in combination with either glucose or fructose. All of the Maillard reaction solutions without S9 mix induced significant increases in chromosome aberrations in CHO cells (Fig. 5). Mitotic recombination and mutation occurred in Saccharomyces cerevisiae strain D5 cells when exposed to all Maillard reaction solutions. When Salmonella TA 100 cells were treated with Maillard reaction solutions without S9 activation, induction of revertants to histidine prototrophy occurred (Fig. 6). Mutagenic activity of the browning reaction solutions increased with a rise in pH from 7 to 10.

Shinohara et al. (26) presented a dose-response curve for the mutagenic activity of a lysine–glucose solution heated at 100°C for 10 hr when the Ames test (TA 100 strain) was used without S9 mix. The number of revertants increased to about 750 per plate over a narrow dose range and thereafter the doses were lethal to the Salmonella. It is of interest to note that the revertant numbers increased with a rise in brown color intensity of the reaction solution.

Several fractions isolated by Mihara and Shibamoto (27) from a heated (100°C for 2 hr) cysteamine–glucose model system possessed mutagenic activity which was assessed by the Ames test using strain TA 98 and TA 100 with and without microsomal S9 activation. Cysteamine, a decarboxylated compound of cysteine, presumably appears after the formation of Schiff's bases during the Maillard reaction in cysteine-containing food products. In a methylene chloride extract of the heated cysteamine–NH₂OH system, thiazolidine and 2-methylthiazolidine were identified and found to possess mutagenic activity. The aqueous nonvolatile fraction possessed a fairly strong mutagen identified as 2-(1,2,3,4,5'-pentahydroxy)-n-pentythiazolidine. Some unidentified compounds in the heated cysteamine-glucose system suppressed mutagenic activity.

Since Maillard reactions are ongoing in stored reducing sugar–protein systems, mutagens should be formed. However, Pintauro et al. (28) found no mutagenic activity in the lipid- and water-soluble fractions of an egg albumin–glucose mixture stored at 37°C for up to 40 days. The samples were assessed by the Ames mutagenicity test.

Wei et al. (29) heated a starch–glycine mixture at 290°C for 40 min to simulate baking conditions. The combined mixture of volatile reaction compounds exhibited a dose-related mutagenicity towards strain TA 98 after metabolic activation with S9. On the other hand, the combined volatiles of starch heated alone did not possess mutagenic activity.

Several studies have indicated that products of the Maillard reactions are mutagenic. Bjeldanes and Chew (30) showed that several 1,2-dicarbonyl compounds, such as diacetyl and maltol, displayed weak mutagenic

**Figure 6.** Mutagenicity of heated fructose-amino acids as assessed by the Ames/Salmonella test (25): (●) initial pH 7; (▲) initial pH 10.
activity with TA 100 in the Ames mutagenicity assay. Dihydroxyacetone, glyceraldehyde, glyoxal, methyl glyoxal, and glyoxylic acid were found by Yamaguchi and Nakagawa (31) to be mutagenic towards TA 100, but the mutagenicity of these compounds was reduced by S9 treatment. DNA was degraded in the presence of these trioses and glyoxal derivatives.

Three short-term assays were employed by Stich et al. (32) to examine pyrazine and four of its alkyl derivatives (2-methylpyrazine, 2-ethylpyrazine, 2,5-dimethylpyrazine, and 2,6-dimethylpyrazine) for mutagenicity. Exposure of Salmonella organisms to these compounds did not result in the induction of revertants to histidine prototrophy. However, with Saccharomyces cerevisiae strain D5, aberrant colonies increased after exposure to each of the pyrazine compounds. Pyrazine and its derivatives induced a significant (7- to 57-fold) increase in the frequency of chromosome aberrations (breaks and exchanges) in CHO cells.

Caramelize reactions may be involved in the production of mutagens during the heat processing of food. Stich et al. (24) heated a variety of sugars (sucrose, fructose, glucose, mannose, maltose, and arabinose) at 180°C for 1 hr to bring about caramelize. CHO cells were exposed to aqueous solutions of the caramelize sugars and then clastogenicity was evaluated. Each of the caramelize sugars induced a high frequency of chromosome breaks and exchanges in the treated CHO cells whereas unheated sugars did not increase the frequency of chromosome aberrations.

According to Okamoto et al. (33), carbohydrate pyrolysates produced by heating mono- and disaccharides at 700°C enhanced the mutagenicities of 2-acetylaminofluorene (2-AAF), tryptophan pyrolysate, (Trp-P) and benzo(a)pyrene (BP) with TA 98 and TA 100 strains in the presence of S9. Pyrolysis of carbohydrates at temperatures lower than 600°C resulted in progressively lower mutagenic enhancement. Sucrose pyrolysate had the highest enhancing effect. The number of his* revertants induced by 2-AAF were increased 10-fold in the presence of sucrose pyrolysate heated at 700°C. By itself, sucrose pyrolysate had a low mutagenic activity.

Furan and furan derivatives have been identified as compounds in caramelize sugars. Stich et al. (34) investigated the clastogenic activity of furans and some derivatives. CHO cells were exposed for 3 hr to solutions of 2-methylfuran, 2,5-dimethylfuran, furfural, 5-methylfurfural, furfuryl alcohol, and 2-furyl methyl ketone. For furan derivatives with no S9 treatment, the frequency of metaphase plates with chromosome aberrations increased with a rise in chemical concentration. The parent compound furan without S9 treatment had no clastogenic activity. When furan derivatives were treated with a S9 mix, the clastogenic activities of 5-methylfurfural, 2-furyl methyl ketone, furfuryl alcohol and furfural increased, while those of 2-methylfuran and 2,5-dimethylfuran decreased.

### Antimutagenic Properties of Browning Reaction Systems

The concept of food components being protective against cancer initiation and promotion has gained considerable attention from cancer researchers and food scientists. Results of epidemiological, animal, and short-term assay studies suggest that naturally occurring antioxidants such as tocopherols, phenolic compounds, and ascorbic acid inhibit the carcinogenesis and mutagenesis (35,36).

Wattenberg (36) classified inhibitors by sequence points at which they act in the carcinogenic process. Some inhibitors prevent the formation of carcinogens, while others, termed blocking agents, prevent carcinogens or mutagens from reaching or reacting with critical target sites. A third group of inhibitors, called suppressing agents, are effective when fed subsequent to administration of carcinogens.

Since large amounts of browning reaction compounds are consumed as dietary components, the antimutagenic properties of browning reaction systems have been explored. Chan et al. (37) used the Ames/Salmonella assay to determine the antimutagenic effect of heated lysine–fructose solution (121°C for 1 hr) and caramelized sucrose (180°C for 1.5 hr). Known potent carcinogens, aflatoxin B1 (AFB1) and N-methyl-N′-nitro-N-nitrosoguanidine (MNNG), were selected as the mutagens. The Maillard reaction system and caramelized sucrose had inhibitory effects on the mutagenicity of AFB1 and MNNG when the Salmonella organisms were exposed to each mutagen in solution with a browning reaction system.

Powrie et al. (38) found that coffee brew possessed a strong inhibitory effect on the mutagenicity of S9-treated AFB1. Such antimutagenic activity of coffee brew may be attributable in part to the binding of AFB1 and metabolites to the melanoidin. The roasting of green coffee beans brings about the formation of numerous Maillard reaction products that include melanoidin (39). According to Molund (40), melanoidin is present in the water-insoluble fraction (WIF) of instant coffee brew.

Molund et al. (41) carried out studies to assess the inhibitory effect of WIF isolated from reconstituted instant coffee and of a model-system melanoidin (MSM) on the mutagenicity of AFB1, MNNG, and benzo(a)pyrene (BP) in aqueous dispersion. The MSM was isolated from a heated glucose–lysine solution. Again the short-term Ames/Salmonella test was used to assess the inhibitory effect of WIF and MSM on carcinogen-induced mutagenesis. Salmonella strain TA 100 was selected as the most suitable organism. About 95% inhibition of AFB1 mutagenicity resulted when WIF was added at a level of 10 mg/mL of treatment medium, whereas MSM at the same concentration level had around 50% inhibitory effect. With respect to MNNG, both WIF and MSM possessed similar inhibitory ability between levels of 3 and 50 mg/mL treatment medium.
At the 25 mg/mL concentration level, WIF and MSM inhibited the MNNG mutagenicity almost completely. When the concentration of either WIF or MSM was increased from 2 to 50 mg/mL, the inhibition of BP-induced mutagenicity rose progressively to about 95%.

WIF isolated from reconstituted instant coffee was found by Molund et al. (42) to be very effective in binding two carcinogens, BP and AFB1, in aqueous dispersion. By using a centrifuge method to sediment WIF and carcinogen–WIF complexes from the carcinogen, the level of binding of BP and AFB1 to WIF was estimated. In BP–WIF dispersions at pH values of 2 and 3, about 80% of BP was bound to WIF whereas approximately 65% was bound when the pH levels of the dispersions were between 4 and 9. Binding of AFB1 by WIF in dispersions with pH values between 2 and 9 was in the vicinity of 50% of added carcinogen. The binding of BP with WIF and with naturally occurring WIF in reconstituted instant coffee brew was examined by separating the BP–WIF complex from unbound BP on Sephaeryl S-1000 and Sephadex G-50 columns. Even with the exposure of the BP–WIF complex to extensive adsorptive surfaces of the gel filtration particles, a considerable amount of BP remained in the bound form.

REFERENCES

1. Dagenskov, M. Time-temperature relationships in industrial cooking and frying. In: Physical, Chemical and Biological Changes in Food Caused by Thermal Processing (T. Huyen and O. Kvala, Eds.), Applied Science Publishers, London, 1977, pp. 77–100.

2. Bengtsson, N. W., Jakobsson, B., and Dagenskov, M. Cooking of beef by oven roasting: a study of heat and mass transfer. J. Food Sci. 41: 1047–1053 (1976).

3. Skjoldebrand, C., and Olsson, H. Crust formation during frying of minced meat products. Lebensm. Wiss. Technol. 13: 48–50 (1980).

4. MacLeod, G., and Seyyed-Ardabili, M. Natural and simulated meat flavors. In: CRC Crit. Rev. Food Sci. Nutr., Vol. 14. (T. E. Furia, Ed.), CRC Press, Boca Raton, FL, 1981, pp. 309–437.

5. Feeney, R. E., and Whitaker, J. R. The Maillard reaction and its prevention. In: Food Protein Deterioration (J. P. Cherry, Ed.), American Chemical Society, Washington, DC, 1982, pp. 201–229.

6. Hurrell, R. F. Maillard reaction in flavor. In: Food Flavors: Part A. Introduction (I. D. Morton and A. J. MacLeod, Eds.), Elsevier Scientific, Amsterdam, 1982, pp. 399–437.

7. Waller, G. R., and Feather, M. S. The Maillard Reaction in Foods and Nutrition. American Chemical Society, Washington, DC, 1983.

8. Spark, A. A. Role of amino acids in nonenzymic browning. J. Sci. Food Agr. 29: 303–316 (1969).

9. Schallenberger, R. S., and Birch, G. G. Sugar Chemistry. AVI Publishing Co., Westport, CT, 1975.

10. Hodge, J. E., and Osman, E. M. Carbohydrates. In: Principles of Food Science, Part I, Food Chemistry (O. Fennema, Ed.), Marcel Dekker, New York, 1976, pp. 41–138.

11. Motai, H. Relationship between the molecular weight and the color intensity of color components of melanoidins from glycine–xylose system. Agr. Biol. Chem. 38: 2299–2304 (1974).

12. Benzing-Purdie, L., and Ripmaster, J. A. Melanoidins and soil organic matter: evidence of strong similarities revealed by 13C CP-MAS NMR. Soil Sci. Soc. Am. J. 47: 56–60 (1983).

13. Korzhova, L. P., and Romakov, Y. A. Reaction dynamics and properties of melanoids formed by the glyceraldehyde-glycine reaction. Prikl. Biokhim. Mikrobiol. 15: 585–598 (1979).

14. Hruby, T. A. Spectroscopic study of the reaction products of dihydroxy-acetone with amino acids. J. Soc. Cosmet. Chem. 28: 25–35 (1977).

15. Gomyo, T., and Horikoshi, M. On the interaction of melanoid with metallic ions. Agr. Biol. Chem. 40: 35–40 (1976).

16. Horikoshi, M., and Gomyo, T. Precipitation of protein in the presence of melanoidins. Agr. Biol. Chem. 40: 1311–1317 (1976).

17. Hryn, C. F., and Carpenter, K. J. The reactive lysine content of heat-damaged material as measured in different ways. Brit. J. Nutr. 32: 589–604 (1974).

18. Lee, C. H., Sherr, B., and Koh, Y. Evaluation of kinetic parameters for a glucose-lysine Maillard reaction. J. Agr. Food Chem. 32: 379–382 (1984).

19. Willits, C. O., Underwood, J. C., Leto, H. G., and Ricciuti, C. Browning of sugar solutions. I. Effect of pH and type of amino acid in dilute sugar solutions. Food Res. 23: 61–67 (1958).

20. Lewis, V. M., and Lea, C. H. A note on the relative rates of reaction of several reducing sugars and sugar derivatives with casein. Biochim. Biophys. Acta 4: 532–534 (1959).

21. Lea, C. H., and Hanan, R. S. The effect of activity of water, of pH and of temperature on the primary reaction between casein and glucose. Biochim. Biophys. Acta 3: 313–325 (1949).

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

23. Toda, H., Sekizawa, J., and Shibamoto, T. Mutagenicity of the L-rhamnose–ammonia–hydrogen sulfide browning reaction. J. Agr. Food Chem. 29: 381–384 (1981).

24. Stich, H. F., Stich, W., Rosin, M. P., and Powrie, W. D. Clastogenic activity of caramel and caramelized sugars. Mutat. Res. 91: 129–135 (1981).

25. Powrie, W. D., Wu, C. H., Rosin, M. P., and Stich, H. F. Clastogenic and mutagenic activities of Maillard reaction model systems. J. Food Sci. 46: 1433–1438 (1981).

26. Shinohara, K., Wu, R.-T., Jahan, N., Tanaka, M., Morinaga, N., Murakami, H., and Omura, H. Mutagenicity of the browning mixtures of amino-carbonyl reactions on Salmonella typhimurium TA100. Agric. Biol. Chem. 44: 671–672 (1980).

27. Mihara, S., and Shibamoto, T. Mutagenicity of products obtained from cysteamine-glucose browning model systems. J. Agr. Food Chem. 28: 62–66 (1980).

28. Pintauro, S. J., Page, G. V., Solberg, M., Lee, F. C., and Chichester, C. O. Absence of mutagenic response from extracts of Maillard browned egg albumin. J. Food Sci. 46: 1442–1443 (1980).

29. Watanabe, M., I., Kitamura, F., Ozawa, S., and Shibamoto, T. Mutagenicity of Maillard browning products obtained from a starch-glucose model system. Food Cosmet. Toxicol. 19: 749–751 (1981).

30. Bjeldanes, L. F., and Chew, H. Mutagenicity of 1,2-dicarbonyl compounds: maltol, kojic acid, diacetly, and related substances. Mutat. Res. 67: 367–371 (1979).

31. Yamaguchi, T., and Nakagawa, K. Mutagenicity of and formation of oxygen radicals by triose and glyoxal derivatives. Agr. Biol. Chem. 47: 2461–2465 (1983).

32. Stich, H. F., Stich, W., Rosin, M. P., and Powrie, W. D. Mutagenic activity of pyrazine derivatives: a comparative study of Salmonella typhimurium, Saccharomyces cerevisiae and Caenorhabditis elegans. J. Nutr. 110: 581–584 (1980).

33. Okamoto, H., Mizusaki, S., Yoshida, D., and Matsumoto, T. Enhancing effect of carbohydrate pyrolysatres on mutagenesis in Salmonella typhimurium. Agr. Biol. Chem. 32: 1433–1438 (1979).

34. Stich, H. F., Rosin, M. P., Wu, C. H., and Powrie, W. D. Clastogenicity of furans found in food. Cancer Letters 15: 89–96 (1981).

35. Rosin, M. P. Inhibition of genotoxic activities of complex mixtures by naturally occurring agents. In: Carcinogens and Mutagens in the Environment, Vol. I. Food Products (H. F. Stich, Ed.), CRC Press, Boca Raton, FL, 1982, pp. 259–273.

36. Wattenberg, L. W. Inhibition of neoplasia by minor dietary con-
stituents. Cancer Res. (Suppl.) 43: 2448–2453 (1983).
37. Chan, R. I. M., Stich, H. F., Rosin, M. P., and Powrie, W. D. Antimutagenic activity of browning reaction products. Cancer Letters 15: 27–33 (1982).
38. Powrie, W. D., Wu, C. H., and Stich, H. F. Unpublished data, 1985.
39. Clifford, M. N. The composition of green and roasted beans. Process Biochem. 17: 13–19 (1975).
40. Molund, V. P. Inhibition of carcinogen-induced biological responses with a coffee water-insoluble portion and a model-system melanoidin. Ph. D. thesis. University of British Columbia, Vancouver, B.C., Canada, 1985.
41. Molund, V. P., Powrie, W. D., and Stich, H. F. Inhibition of carcinogen-induced mutagenesis by the water-insoluble fraction of coffee brew and a model-system melanoidin. Can. Inst. Food Sci. Technol., submitted.
42. Molund, V. P., Powrie, W. D., and Stich, H. F. Binding of benzo(a)pyrene and aflatoxin B₁ by the water-insoluble fraction from coffee brew. Can. Inst. Food Sci. Technol., submitted.