Effects of Meat Composition and Cooking Conditions on Mutagen Formation in Fried Ground Beef

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The influence of meat composition and conditions of cooking on mutagen formation in fried ground beef was examined. Ground beef patties or reconstituted samples prepared from dehydrated and defatted meat were fried for various times (2–20 min/side) at one or a series of temperatures (150–300 °C) on stainless steel or other cooking surfaces. The mutagenicity of the exterior portion was determined with *Salmonella* strain TA 1538. Weight loss, water content in the outer portion, and reflectance of the surface of the cooked meat were also determined. The metallic and Teflon surfaces produced similar levels of mutagenicity in samples cooked under similar conditions. The ceramic and enamel surface appeared to retard frying rates with correspondingly reduced levels of mutagenicity. Mutagen production was strongly dependent on water content of the reconstituted patties above 40%. Mutagenicity was nearly independent of fat content with the totally defatted samples producing maximum mutagenicity in this series.

Transformation of food components to mutagenic substances is reported to occur under a wide variety of conditions. Mutagens soluble in aqueous acid result from high-temperature (greater than 300 °C) pyrolysis of proteins and amino acids (Matsumoto et al., 1978, 1977). Basic mutagens are also produced by extended low-temperature (less than 100 °C) treatments of aqueous meat mixtures (Dolara et al., 1979). Normal cooking of meats generally requires conditions between these extremes. Whereas basic mutagens are produced in meat under normal frying conditions (Felton et al., 1981; Spingarn and Weisburger, 1979; Pariza et al., 1979), the full identities of these substances and their relation to substances produced under the low- and high-temperature conditions remain to be elucidated.

The present study was undertaken to examine in detail the effect of cooking conditions and meat composition on mutagen production in ground beef, the most important source of meat protein in the U.S. diet (Plumlee et al., 1981). An objective of the study was to determine the physicochemical parameters which could be useful in understanding the chemical processes involved in mutagen production. This information could be used to specify cooking conditions which will result in an acceptable product with minimal mutagen content.

MATERIALS AND METHODS

Meat. Ground beef round (15% average measured fat content) was purchased at local markets and cooked in patties (100 g, ~11 cm in diameter × 1 cm). The outer 3 mm of both sides was removed and extracted for bioassays. Results of previous studies have shown the mutagenic activity to reside in this portion (Felton et al., 1981).

Patties of variable water and fat content were prepared from freeze-dried meat. The weight fraction of water removed was 66%. Appropriate amounts of distilled water were added back for the variable water content experiment. Defatted meat was prepared by petroleum ether (30–60 °C) extraction (15 h) of freeze-dried material in a Soxhlet apparatus. Petroleum ether was removed in vacuo and the remaining oil was used to reconstitute the meat samples, which were also reconstituted to their original water content. Reconstituted meat samples had the compositions indicated in Figures 1 and 2. Compositions of uncooked, reconstituted samples and fried portions were determined by prescribed methods (Joslyn, 1970).

Cooking Procedures. Unless otherwise indicated, meat was fried on an electric, stainless steel griddle (Model EL124, 6 kW, 240 V, Cecilware Corp., Long Island City, NY). Temperatures of the frying surface and meat were monitored by a multipoint industrial recorder (Model Speedomax, Leeds and Northrup, North Wales, PA) and controlled automatically.

In experiments to determine the effect of cooking surface on mutagen production, four patties were cooked on each surface (either a commerical pan or a sheet of metal) (Table I) heated on the Cecilware griddle. Heat transfer from griddle to cooking surface was maximized with an intervening salt or Wood's metal bath. The temperature of all surfaces was initially 200 °C. Temperatures were

Table I. Mutagenicity of Organic Base Fraction from Ground Beef Fried on Different Surfaces at 200 °C for 6 min/Side

	no. of expt	revertants[a]	% weight lost during cooking	% moisture content of outer layer
stainless steel (control)	5	6270 ± 1200[b]	40.2 ± 3.0	45.4 ± 0.4
stainless steel on aluminum	1	7160	36.5	47.0
aluminum	1	4300	38.1	45.8
Teflon	4	4250 ± 2370	36.6 ± 4.2	51.5 ± 3.4
cast iron	1	3550	40.5	46.3
enamel	1	1425	30.4	54.1
ceramic	2	900 ± 85	30.8 ± 0.8	53.0 ± 1.2

[a] For Table I and for Figures 1-3, five mutation frequencies per 100 g equiv in *Salmonella* strain TA 1538 were determined by regression analysis from the linear portion of dose-response curves. For a single-dose response curve the standard deviation of the regression slope is less than 4% of the slope. The average variation of slope between replicate assays on the same sample of organic base fraction is 6%. The coefficient of variation of complete replications of cooking, extracting, and assaying of batches of ground beef (stainless steel, above) is 19%. [b] Standard deviation of estimates from n experiments.

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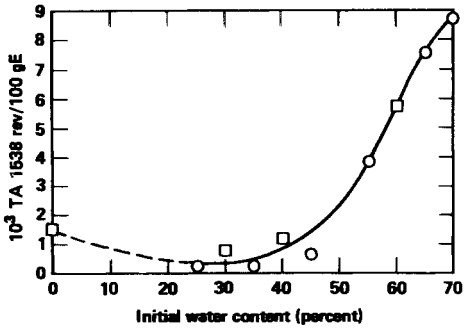

Figure 1. Effects of variation of initial water content on mutagenicity of ground beef. Samples are fried at 200 °C for 6 min/side, and the extracted basic fraction is tested for activity in the *Salmonella* mutagenesis assay. (□) and (○) are data from two separate experiments. The original fat to protein ratio of approximately 0.8 in the ground beef was maintained in these samples.

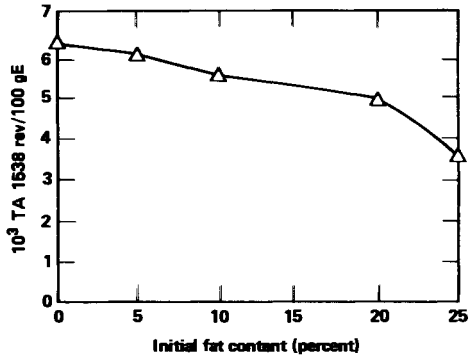

Figure 2. Effects of variation of initial fat content on mutagenicity of ground beef. Samples are fried at 200 °C for 6 min/side, and the extracted basic fraction is tested for activity in the *Salmonella* mutagenesis assay. An amount of water equal to that removed in freeze-drying was added to each sample. Graded amounts of the extracted fat were added to the reconstituted samples before cooking.

maintained as close as possible to 200 °C with an Omega (Model 802 M) controller connected to a thermocouple. The thermocouple measured the temperature at the cooking surface/meat interface. Patties were cooked 6 min/side, the outer cooked layers were removed, and the extracted organic bases were assayed for mutagenic activity.

In one experiment, ground beef patties were cooked in batches for 2, 4, 6, 10, 15, and 20 min/side at 150, 200, 250, and 300 °C on the stainless steel griddle.

All cooked samples were assayed for weight loss, water content in exterior portion, reflectance, and mutagenicity.

Extraction Procedures. The acetone extraction method described previously (Felton et al., 1981) was used for all meat samples.

Mutagenesis Assays. Mutagenicity of basic extracts was determined with *Salmonella* tester strain TA 1538 by the method of Ames et al. (1975), as described by Felton et al. (1981). The bacterial strain was provided by B. N. Ames (Berkeley, CA). The induced mutation frequencies per 100 g equiv (gram equivalents; indicates fresh weight of meat from which extracts were obtained) of meat were determined by regression analysis from the linear portions of dose–response curves.

Reflectance Measurements. Color measurements of fried meat were made on a tristimulus reflectance colorimeter (Colormaster Model V, MEECO, Warrington, PA). Percent red reflectance was recorded in three areas on each side of four patties for each time–temperature combination

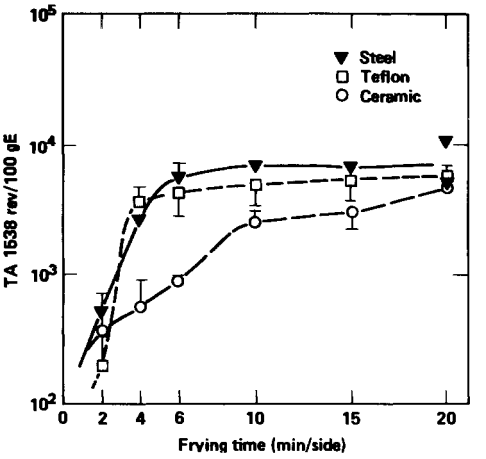

Figure 3. Effect of cooking surface on mutagenicity of fried beef. All cooking surfaces were equilibrated to 200 °C before cooking. Error bars are ±1 standard deviation, shown in one direction only.

examined. Thus, each value is the mean value calculated from 24 readings. Measurements were made within 24 h of frying whenever possible. All measurements were made at room temperature.

RESULTS

Meat Composition. Results indicating the influence of meat composition on mutagenicity of fried samples are summarized in Figures 1 and 2. Data presented in Figure 1 were derived from two separate experiments. Clearly, mutagen production is strongly dependent on the amount of moisture initially present in the meat above approximately 40%. Below this level mutagen production is low and appears independent of moisture level.

The relative fat content of meat appears to have little effect on mutagenicity of cooked samples. Data presented in Figure 2 indicate maximum mutagenicity of defatted samples with a slight decrease in activity with increasing fat content. Linear regression calculation of these data indicate a negative slope, which is significantly different from zero ($P = 0.01$).

Cooking Surface. The mutagenic activities of the basic fraction from hamburger cooked on the different surfaces are shown in Table I. Meat cooked on aluminum, cast iron, stainless steel, and Teflon surfaces contained the highest activity. Meat cooked on the ceramic and enamel surfaces contained less mutagenic activity. In this table also shown are the weight loss during cooking and final moisture content of the outer layer. The least mutagenic samples (ceramic and enamel) showed the smallest weight losses during cooking. These samples also showed the greatest moisture content in the outer layer. However, for the limited number of cases where replicate measurements are available, these differences in weight loss and moisture were not statistically significant.

A more detailed investigation of the effects of cooking surface on mutagen production was conducted by measuring mutagenic activity of meat cooked at 200 °C for various times on Teflon, ceramic, and stainless steel surfaces. The results are indicated in Figure 3. Mutagen production on the stainless steel and ceramic surfaces was reproducible with lower activity observed in samples cooked on the ceramic surface. Mutagen production on the Teflon surface was somewhat variable between experiments. However, the overall trend of mutagenicity in samples fried for various times on Teflon was similar to the trend for samples fried on stainless steel. Weight loss, crust moisture loss (data not shown), and reflectance (Figure 4) are consistent with a slower rate of cooking for

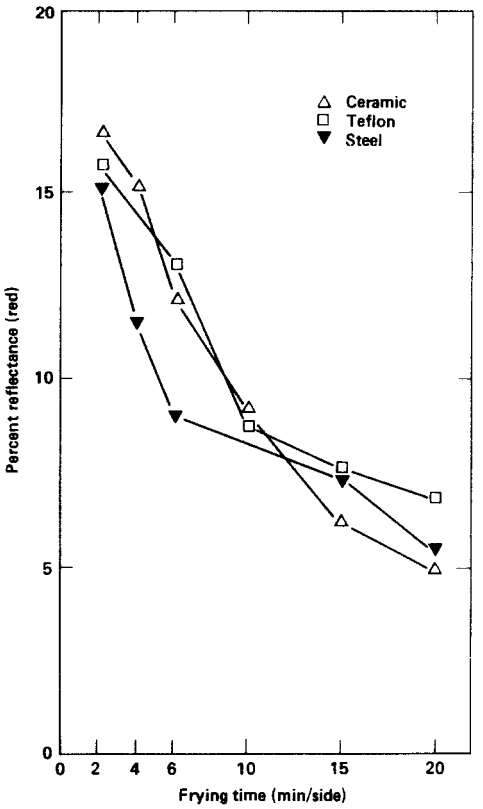

Figure 4. Effect of frying surface on reflectance loss in ground beef. Initial frying temperature of all surfaces was 200 °C.

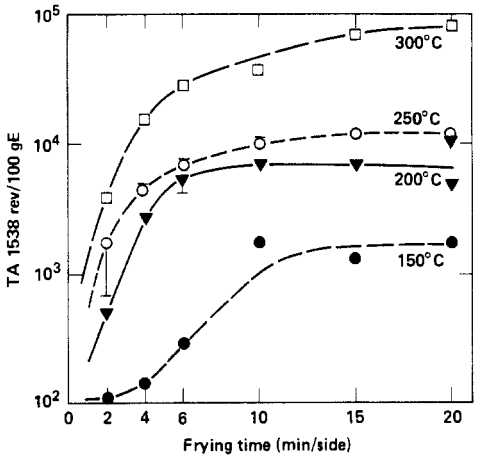

Figure 5. Effects of time and temperature of frying on mutagenicity of ground beef. Standard-sized patties were fried on a stainless steel griddle preequilibrated at the stated temperatures, and the basic extract was assayed for mutagenicity. Error bars are ±1 standard deviation, shown in one direction only.

samples fried on a ceramic surface relative to those fried on stainless steel. Weight loss for meat cooked on the Teflon surfaces was only slightly slower than on stainless steel; however, the reflectance data more closely resembled those on a ceramic surface.

Time–Temperature Effects. The induced mutation frequencies from the time–temperature study of meat fried on stainless steel are given in Figure 5. At 200, 250, and 300 °C, induced mutagenicity increases rapidly until about 10 min/side of cooking, followed by only a moderate increase (300 °C samples) or no further increase in mutagenicity at longer times. Additional parameters determined on the cooked meat were weight loss, percent water in the exterior portion, and red reflectance (Figure 6) of surfaces.

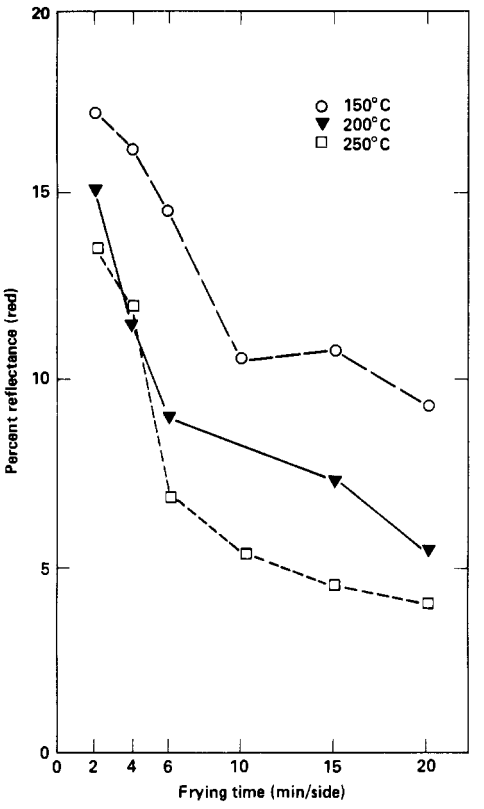

Figure 6. Effect of frying time and temperature on reflectance loss in ground beef. Samples were fried on the stainless steel griddle preequilibrated at the stated temperatures.

The dependence of mutagen formation on time and temperature was determined by multiple linear regression. Over the 150–300 °C range studied, mutation frequency (M_f) was found to be related to temperature (in K, T) and time (min, t) by the expression

$$M_f = 2.8 \times 10^{-31} t^{1.17} T^{12.3} \qquad (R^2 = 0.92)$$

Mutagen formation is a multiplicative function of time and temperature; however, the very high exponent for temperature indicates its predominant role. Inclusion of weight loss as an additional independent variable did not appreciably improve the regression (data not shown). This appears to result from the fact that weight loss is proportional to temperature only at ≥10 min/side of cooking, when mutagen formation has nearly reached a maximum.

DISCUSSION

The results of these studies indicate that mutagen production in ground beef depends on a complex interaction of composition of the meat, cooking surface characteristics, and time and temperature conditions of the cooking.

The effects of variation of water and fat content on mutagenicity of fried meat suggest that adequate water is required (~40% by weight), perhaps as a heat-transfer agent or as a reaction medium for water-soluble intermediates. The observed independence of mutagen production and content of fat, an excellent heat-transfer agent, suggests that water may be serving the latter purpose. Whereas the requirement of water as a reactant in mutagen formation cannot be ruled out, based on the present results, fat is clearly not required as a reactant. The results provide evidence that increased fat levels may even lead to a slight decrease in mutagenicity in fried meat possibly due to a loss of mutagens into the cooking juices.

The present results appear to be at odds with the results of others who have reported low mutagenicity of low-fat

meat (Spingarn et al., 1981). However, the present study was carried out with reconstituted samples from totally dehydrated and defatted meat. The consistency of the reconstituted samples was similar to, but certainly different from, the consistency of ground beef. These physical effects of total dehydration and defatting may contribute to the differences in the observed results using reconstituted and normal low-fat ground beef.

Evidence from the cooking surface experiments suggests that there is not likely to be a specific role of surface metal in catalysis of mutagen production. The general indications from data presented in Table I are that all the metal surfaces and the Teflon surface produce similar levels of activity in fried samples. The consistently lower activity produced on the ceramic and enamel surfaces can be ascribed to the reduced cooking rate for these samples. Thus, all the parameters measured for the samples cooked on the ceramic surface indicate, for example, that after 10 min of cooking at 200 °C on this surface, samples have the total weight loss (~40%), crust moisture (~45%), and red reflectance (~9%) of samples fried for 6 min on stainless steel at the same temperature setting. Mutagenicity of the 10-min ceramic samples (~4000 revertants/100 g equiv), while somewhat less, is also in the range of activity of the 6-min stainless steel sample (~5600 revertants/100 g equiv). A possible advantage of the ceramic surface in minimizing mutagen production during frying is that, at a given temperature setting, rates of cooking and mutagen production are lower on the ceramic surface and therefore can be more easily controlled.

The results of the Teflon experiments provide evidence that the usual indicators of degree of cooking cannot always be relied on to give consistently accurate estimates of the mutagenicity of fried meat. For example, samples fried for 6 min/side at 200 °C on Teflon and stainless steel showed similar total weight losses (~40%) and crust moisture (~47%). The appearance of the samples and the red reflectance data suggest that the 6 min/side Teflon samples looked more like the 2–4 min/side stainless steel samples. Nevertheless, the Teflon samples were nearly as mutagenic (~4300 revertants/100 g equiv) as the 6 min/side stainless steel samples (~5600 revertants/100 g equiv).

The complex nature of the frying process considerably complicates interpretation of kinetic information. Thus, for example, observed rates of mutagen production may be affected by exhaustion of substrate, vaporization (Rappaport et al., 1979) or destruction of mutagen, or formation of a dense crust with inhibition of further heat transfer. Nevertheless, the results of the present study indicate that browning of meat patties, as indicated by loss of red reflectance and visual appearance, may occur without production of mutagenic activity. Thus, meat cooked for as long as 20 min/side at 150 °C on stainless steel appeared as well done (dark brown-black) as samples fried for 6 min/side at 200 °C on stainless steel, but the former samples were considerably less mutagenic (~1700 revertants/100 g equiv) than the latter (~5600 rev/100 g equiv). These findings agree with the preliminary findings reported by others (Pariza et al., 1979).

The results presented in Figures 3 and 5 of frying time–temperature experiments do not provide evidence for a general lag period of cooking before mutagen production becomes readily detectable. A lag period of approximately 4 min has been reported for mutagen production in meat patties fried by a process which involved turning patties at 1-2 min intervals (Pariza et al., 1979). Our frying procedure incorporated a single turn of the patty during heating, a technique which appears more closely to approximate the conventional cooking process. The latter technique, by allowing a more continuous heating of one side of the patty than the multiple turn method results in more rapid cooking on one side and yields high levels of mutagenicity early in the cooking process. A lag in mutagen formation exceeding 5 min of frying time was also reported by Spingarn and Weisburger (1979). In these experiments timing was apparently initiated when heating of the frying surface was begun, whereas in our experiments the meat was applied to a preheated surface. In our experience mutagen formation is rapid when meat surface temperatures rise substantially above 100 °C.

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Registry No. H_2O, 7732-18-5; Al, 7429-90-5; Fe, 7439-89-6; Teflon, 9002-84-0; stainless steel, 12597-68-1.

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