EFFECT OF FAT CONTENT ON MUTAGENICITY OF FRIED BEEF

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INTRODUCTION

The role of the lipid fraction in the development of mutagenic activity of meat is still not very clear. Barnes et al. (1983) and Barnes & Weisburger (1983, 1984) claimed that fat is important in the formation of the mutagenic compounds. Barnes et al. (1983) developed a quantitative assay for 2-amino-3-methyl-imidazo[4,5f]-quinoline (IQ) based on thin layer chromatography and high performance liquid chromatography. Using this method, high fat (25% of total wet weight) and low fat (11%) beef patties, cooked at 5 min/side, were found to contain 20.1 and 0.5 μg of IQ per kg of sample, respectively. Barnes & Weisburger (1983) reported that inclusion of beef lipids into a heated mixture of creatinine, glycine and glucose increased the mutagenic activity three-fold. Barnes & Weisburger (1984) showed that adding either corn oil or beef fat (beef suet) increased the mutagenic activity of fried ground beef. Both of these lipids doubled the amount of mutagens formed in fried meat when added to the samples at a concentration of 20% based on the wet weight of the ground beef. Barnes & Weisburger (1984) showed the addition of glycine and creatinine to ground beef prior to cooking enhances mutagen formation by approximately 50%. On adding glycine, creatinine and glycerol to the system, mutagen formation increased by approximately 100%. These results indicate that lipid decomposition may contribute precursor(s) for mutagen formation and that glycerol may account for at least part of the mutagen-enhancing effect of fat.

However, Felton et al. (1984) and Knize et al. (1985) claimed that the increased mutagenic effect was not due to the fat *per se* but rather due to increased heat penetration associated with the increase in fat content. The present study was designed to evaluate the role of fat in the development of mutagenic activity of fried ground meat.

METHODS

Lean ground beef (1.8% fat) was thawed and divided into five composite aliquot samples. Each sample was mixed with a different amount of chopped frozen beef fat in order to give a final fat content 2, 4, 8, 12 and 18% in the sample. Three subsamples were taken from each aliquot for fat and moisture analyses. Then, 500 g of sample was made into 100g patties about 0.5 cm in thickness, which were used for frying, extraction and determination of mutagenicity.

Frying Method

A stainless steel, Teflon-coated electric fry pan was used for the frying procedure. The temperature control of the fry pan was set at 215°C. Before frying, the fry pan was preheated for 5 min. In the

present study, the patties were fried for 6 or 9 min per side.

Extraction Method

The fried patties were extracted using modifications of the methods of Pariza et al. (1979) and of Felton et al. (1981). The fried ground beef patties were homogenized in 3 volumes of distilled water with a Waring Blender. The homogenate was filtered through cheese cloth. The solids were again homogenized in 1 volume of acetone and H_2O (1:1, v/v) and filtered as before. The filtrates were combined and cooled at -20°C for 5 min to induce protein precipitation. The combined filtrate was filtered through glass wool. The filtrate was acidified to pH 2 with HCl and extracted 3 times with CH_2Cl_2 to obtain the soluble acidic components in the CH_2Cl_2 extract. The upper aqueous layer was adjusted to pH 12 by adding NaOH and extracted again with CH_2Cl_2 to obtain the basic components. The CH_2Cl_2 extract containing the acidic and basic components were passed through anhydrous Na_2SO_4 to remove the water and then evaporated to dryness under vacuum at 37°C on a rotatory evaporator. The residues were dissolved in 20% methanol in $CHCl_3$ (v/v). The solutions were transferred to vials and dried under a stream of nitrogen gas, after which the residues were dissolved in dimethylsulfoxide (DMSO) and used for the Ames test.

Moisture Content

The A.O.A.C. (1975) procedure for determining moisture was used.

Fat Content

The fat content was determined using the Goldfisch extraction method of the A.O.A.C. (1975).

Ames Test

The Ames test was carried out as described by Ames et al. (1975) and Maron & Ames (1983). Salmonella typhimurium strain TA98 was provided courtesy of Dr. Bruce N. Ames at University of California, Berkeley, CA. After extraction, the basic fraction was tested for mutagenicity using tester strain TA98 + S-9.

RESULTS

The results of the Ames test are shown in Figure 1 and indicate that samples with fat concentrations ranging from 4 to 8% showed the least amount of mutagenicity. At 14% fat there was an approximate doubling of mutagenic activity, while the 18% fat sample had less mutagenic activity than that of the 14% fat sample. These results agree with Knize et al. (1985), who reported that increasing the fat content from 8 to 15% enhanced mutagenicity on cooking at either 180 or 240°C for 6 mins per side. However, it was found in the same study that increasing the fat content from 15 to 30% resulted in a slight reduction in overall mutagenic activity, which is similar to the results in the present study.

On frying the ground beef at 9 mins per side, the mutagenicity decreased directly with fat content (Figure 1). Although the meat fried at 9 mins per side

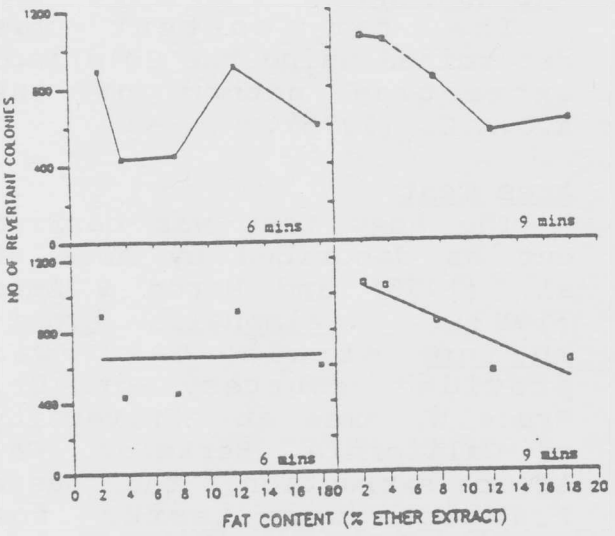

Figure 1.-Relationship between the fat content of ground beef patties and mutagen formation after cooking for either 6 or 9 min per side. Each point represents 6 replicates. The concentration of the basic fraction added was 50g meat equivalents/plate. The lower two graphs present the regression lines of the data shown in the upper two graphs.

showed less variation in mutagenicity than that fried at 6 mins per side, the reason that there is less fluctuation in mutagenicity on frying at 9 mins per side is not clear. A possible explanation is that with longer frying times most of the fat was cooked out of the patties, which would probably concentrate the precursor(s) of mutagen formation and reduce the dilution effects from the fat. These data confirm the fact that fat is not the major contributor of precursor(s) for mutagen formation. This is in agreement with earlier studies by Felton et al. (1984) and Knize et al. (1985), who demonstrated that fat content did not contribute to meat mutagen formation. However, the results are in contrast to studies by Barnes et al. (1983) and Barnes & Weisburger (1983, 1984), who reported that mutagenicity increased directly with fat content.

The regression line (Figure 1) indicates that the fat content of ground beef fried at 6 mins per side did not effect mutagenicity. No doubt, the fluctuation in mutagenicity at different fat concentrations accounts for the low straight line relationship which shows no effect of fat on mutagenic activity. However, on frying at 9 mins per side, the fat concentration was negatively related to mutagenicity indicating that fat does not contribute to mutagen formation. Therefore, the meat mutagenicity is not directely related to the fat content. However, fat may still play some important roles in the formation of antimutagenic components in the acidic fraction in the pan fried meat. Pariza (1986) has isolated and identified a antimutagenic modulator in fried hamburger. The modulator, designated CLA (conjugated linoleic acid), is a derivative of linoleic acid. Pariza (1986) stated that CLA is an effective inhibitor of skin cancer in mice. Besides being found in fried hamburger, Pariza (1986) also found CLA in uncooked beef and some dairy products, especially cheese.

SUMMARY

Results of this study show that meat mutagenicity is not directly related to fat content. Apparently, the mutagenic compounds are produced from heating the nonfatty components in the meat, and fat does not directly influence mutagenicity in fried ground beef.

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