Antioxidant Inhibition of Mutagenicity in Fried Meat

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Introduction

A recent review by Ames (1983) has focused attention not only on naturally occuring mutagens and carcinogens but also upon antimutagens and anticarcinogens that also may be constituents of the food supply. On the basis of epidemiological studies, Doll and Petro (1981) and a report by the National Research Council (1982) concluded that there may be a relationship between dietary habits and cancer risks to mankind.
A number of investigators (Lijinsky and Shubik, 1964; Commoner et al., 1978; Pariza et al., 1979; Kasai et al., 1979; Spingarn et al., 1980) have identified either mutagens or antimutagens or both in cooked meat and fish. Weisburger (1979) has discussed the possible mechanisms by which diet influences the incidence of cancer and reviewed the role of temperature and time of cooking upon development of mutagens and the possible relationship to the etiology of human cancer. Although benzo[a]-pyrene, a potent carcinogen, was isolated from the surface of charcoal broiled beefsteak, Nagao et al. (1977) demonstrated that the heating of proteinaceous foods to high temperatures (>300°C) produced mutagenic activity in excess of that accounted for by the benzo[a]-pyrene content. It was later shown that the basic fraction of tryptophan pyrolysis possessed strong mutagenic activity (Inoui et al., 1981), with several compounds (Trp-P-1 and Trp-P-2) being isolated and showing various degrees of mutagenesis by the Ames test (Takayama et al., 1977). Spingarn et al.(1980) then isolated a component from fried beef that had a molecular weight of 198 and an elemental formula of $C_{11}H_{10}N_4$, which was later identified as being a potent mutagen, 2-amino-3-methylimidazo-[4,5f]quinoline (IQ) by Kasai et al.(1980), who also found another strong and closely related mutagen, 2-amino-3,5-dimethylimidazo-[4,5f]quinoline (Me-IQ) to be present. Since that time a number of laboratories have examined the types of mutagens formed in cooked fish, beef and commercial beef extract (Miller, 1985). At least 10 heterocyclic amines have been identified as being present in cooked beef extracts (Felton et al., 1983; Kinze et al., 1948).
These mutagens can be separated by high performance liquid chromatography and quantitated (Barnes et al., 1983). There is also evidence that both fresh and cooked meat contain antimutagens (Pariza et al., 1979), which may be in the form of dietary antioxidants according to the theory of Ames (1983). Since the mutagenic activity is not present in raw meat but is generated during cooking and/or processing, it appears likely that antioxidants added to meat prior to cooking could aid in blocking mutagen formation. Further support for this idea is found in the fact that Fukayama and Hsieh (1984) have recently shown butylated hydroxytoluene (BHT) to decrease the mutagenic response to aflatoxin B_1 by the Ames test in the presence of S-9 from BHT-treated rats in comparison to S-9 from untreated animals.
The effect of antioxidants on mutagen production in cooked ground beef was first described by Wang et al.(1982), who showed that butylated hydroxyanisole (BHA) successfully reduced the mutagenicity of fried beef. Later, Barnes et al.(1983) showed that BHA can inhibit IQ formation by 40% during cooking of beef.

Objectives

The study reported herein was designed to determine if antioxidants could inhibit mutagen formation during pan-frying of ground beef patties at a temperature of 215°C. These studies concentrate on mutagenic activity measured by the Ames test (Ames et al., 1975; Maron and Ames, 1983) and indicate that certain antioxidants suppress mutagenicity during pan-frying of meat.

Materials and Methods

Ground beef patties were prepared to contain either 5.7 or 10% of fat (ether extract). The patties were made approximately 0.3cm. in thickness and prepared to contain antioxidants at the following levels : (a) Control - no added antioxidants and without corn oil; (b) Control (no antioxidants) with 1ml. of corn oil per 500g of meat; (c) Propylgallate (PG) added in same level of corn oil as treatment b to contain either 0.01 or 0.1% - based on the fat content of the meat; (d) butylated hydroxyanisole (BHA) at same levels as in treatment c; (e) butylated hydroxytoluene (BHT) at same levels as treatment c; and (f) Tenox 4 (20% BHA and 20% BHT dissolved in corn oil) added at 0.02 and 0.2% (w/w) based on the fat content.
Frozen ground beef patties were prepared and fried on an electric, stainless steel, Teflon-coated frying pan. The thickness of the ground beef was about 0.3cm, and temperature control of the frying pan was set at 215°C. The meat was cooked for 9 minutes on each side. The fried patties were extracted using a modification of the method 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 remaining yellow filtrate was concentrated under vacuum on a rotatory evaporator to remove the acetone. 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 pH of the aqueous phase was extracted again with CH_2Cl_2 to obtain the basic components. The two CH_2Cl_2 extracts containing the acidic and basic components were passed through anhydrous Na_2SO_4 to temove 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.
The Ames test was carried out as described by Ames et al.(1975) and Maron and Ames (1983). Salmonella typhimurium TA 98 and TA 100 were kindly supplied by Dr.Bruce N.Ames, University of California, Berkeley, CA. Liver homogenate supernatans (S-9) were prepared from rats treated with polychlorinated biphenyls (Arochlor 1254) to induce activity of the microsomal enzymes responsible for detoxifying various xenobiotics. The preincubation technique of Maron and Ames (1983) was used to assay mutagenic activity. Sodium azide (NaN_3) was used as the positive control for TA 100. 2-Acetlyamino-fluorine (2AF) was used as the positive control for TA 98 and TA 100 along with the S9 fraction.

Results and Discussion

As indicated in the Fig.1, the basic extracts from the meat with or without corn oil (A and B) exhibited potent mutagenicity when tested with both TA 98 and TA 100 containing the S9 fraction. No mutagenicity was detected in the acidic fraction. The specific activity of the basic fraction of the meat for strain TA 98 and TA 100 with S9

activation was 249 revertants/g and 3283 revertants/g, respectively. All the antioxidants tested had an inhibitory effect on the mutagenicity, except for BHT (Fig.1). The basic fraction obtained from beef fried with BHT tended to increase mutagenicity for both TA 98 and TA 100 at both levels tested (0.1% and 0.01% of the fat content) in comparison to control samples. Although the basic fraction of the meat fried with 0.1% BHA (group E) still showed some mutagenicity when tested with TA 100 (10.5 revertants/g equivalent of meat), at the 0.01% level BHA inhibited mutagenicity in the fried meat. However, BHA inhibited mutagenicity at levels of both 0.01% and 0.1% of the fat content when tested with TA 98. This agrees with the results reported by Wang et al.(1982) and Barnes et al.(1983). Tenox 4 (a mixture of 20% BHA and 20% BHT in corn oil) inhibited mutagenicity of the fried beef patties at both levels (0.02 and 0.2%) on testing with both TA 98 and TA 100. PG also inhibited mutagenicity with both TA 98 and TA 100 at both levels of usage (0.01 and 0.1%).

The reason that BHT tends to increase mutagenicity and other antioxidants decrease mutagenicity of the basic fraction of the fried meat are still not known. The fat content may also influence the number of colonies formed. Results shown in Table 1 demonstrate that meat with 10% fat exhibited higher mutagenicity than that with 5.7% fat. Spingarn et al.(1981) also reported that mutagenicity reaches a maximum at 10% added fat and subsequently decreases.

Summary

It was shown that all tested antioxidants (BHA, PG and Tenox 4) inhibited mutagenicity, except for BHT, which tended to increase mutagen formation in both tester strains TA 98 and TA 100. Fried meat containing 10% fat was also shown to be more mutagenic than similarly fried meat containing 5.7% fat. This study clearly demonstrates that several, but not all (BHT), synthetic antioxidants inhibit mutagen formation during cooking of meat.

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Table 1. Mutagenic activities (No. of revertants/g equivalents of meat) of TA 98 and TA 100 with S-9 mix for basic extract of beef patties with different fat contents fried at 215°C[a].

Tester Strain	Fat Content %	
	10%	5.7%
TA 98	249	3
TA 100	3283	7

a) Each value represents six replicates.

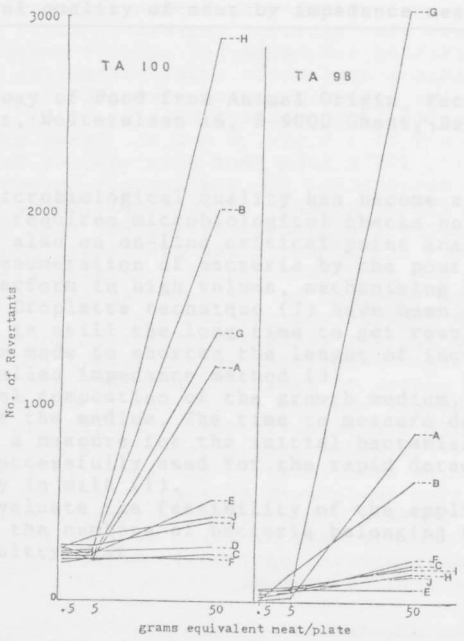

Fig.1. Mutagenic activities of TA 98 and TA 100 with S-9 mix of the basic extract of beef patties (10% fat) fried with different antioxidants (AO). Corn oil (CO) was used as the carrier for AO (1ml/500g). A-No AO nor CO added; B-No AO but CO added; C-50mg PG/500g added; D-5mg PG/500g added; E-50mg BHA/500g added; F-5mg BHA/500g added; G-50mg BHT/500g added; H-5mg BHT/500g added; I-250mg Tenox 4/500g added; J-25mg Tenox 4/500g added.

activation was 289 revertants/g and 3283 revertants/g, respectively. All the antioxidants tested had an inhibitory effect on the mutagenicity, except for BHT (Fig 1). The basic fraction obtained from beef fried with BHT tended to increase mutagenicity for both TA 98 and TA 100 at both levels tested (0.1% and 0.01% of the fat content) in comparison to control samples. Although the basic fraction of the meat fried with 0.1% BHA (group 6) still showed some mutagenicity when tested with TA 100 (10.5 revertants/g equivalent of meat), at the 0.01% level BHA inhibited mutagenicity in the fried meat. However, BHA inhibited mutagenicity at levels of both 0.01% and 0.1% of the fat content when tested with TA 98. This agrees with the results reported by Wang et al (1982) and Barnes et al. (1983). Tenox 4 (a mixture of 20% BHA and 20% BHT in corn oil) inhibited mutagenicity of the fried beef patties at both levels (0.02 and 0.2%) on testing with both TA 98 and TA 100. PG also inhibited mutagenicity with both TA 98 and TA 100 at both levels of usage (0.01 and 0.1%).

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	10%	5.7%
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TA 100	3283	[illegible]

[a] Each value represents six replicates.