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Influence of Commercial Marinades on Heterocyclic Aromatic Amine Formation and Overall Mutagenicity in Fried Beef Steak

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Abstract The effects of commercial marinades were evaluated for their influence on heterocyclic aromatic amine (HAA) formation and overall mutagenicity in fried beef steaks. Three different commercial marinades A, B, and C tested individually reduced the total HAA formation in fried beef steaks by 44, 38, and 40%, respectively. Three different commercial marinades were also effective in reducing the overall mutagenicity in fried beef steaks. There was, however, no significant difference in the inhibition achieved with three different commercial marinades. Reduction of overall mutagenicity was related to the decrease in HAA formation in fried beef steaks.

Keywords: heterocyclic aromatic amine, mutagenicity, marinade, beef

Introduction

When food is cooked, carbonyl and amino compounds react via the Maillard reaction to produce several hundreds of reaction products. Some of these contribute to the color and flavor of the cooked food. The Maillard reaction may also impact the nutritional value of the food. Furthermore, in some cases, the Maillard reaction can lead to the formation of genotoxic compounds called heterocyclic aromatic amines (1). Heterocyclic aromatic amines (HAAs) have been reported to be mutagenic in the Ames Assay, with specific activities of IQ (2-amino-3-methylimidazo [4,5-*f*]-quinoline), MeIQ (2-amino-3,4-dimethylimidazo [4,5-*f*]-quinoline), MeIQx (2-amino-3,8 dimethylimidazo [4,5-*f*]-quinoxaline), DiMeIQx (2-amino-3,4,8-trimethyl-imidazo[4,5-*f*]-quinoxaline), and PhIP (2-amino-1-methyl-6-phenylimidazo [4,5-*b*]-pyridine) ranging from 120 to 661,000 revertants/μg (2, 3). The risk of developing cancer from ingesting HAAs is difficult to calculate, but it may range from 1 per 10,000 to 1 in 50 depending upon the amount of well-done muscle meats ingested and the genetic susceptibility of the person (1).

The precursors of HAAs in cooked meat products are mainly creatine/creatinine, amino acids, and sugars (4). It has been suggested that HAA formation follows the Maillard reaction through the generation of vinylpyrazine, vinylpyridine, and aldehydes (4). Factors influencing HAA formation include temperature, time, and method of cooking, as well as the concentrations of precursors present in the food (5, 6).

It has been reported that concentrations of HAAs or overall mutagenicity in fried ground beef patties can be reduced by the addition of compounds, such as oligosaccharides (7), vitamin E (8), garlic related sulfur compounds (9, 10) soy protein concentrate (11), defatted glandless cottonseed flour (12), and tea polyphenolics (13). Sugar is viewed as a major contributor to HAA formation, but addition of sugars to ground beef patties at levels ranging from 2 to 4 percent reduces HAA formation and overall mutagenicity of cooked ground meat (6). It has been proposed that addition of reducing sugars to meat beyond the optimum needed for formation of HAAs results in formation of Maillard reaction products which inhibit the mechanism of HAA formation (14)

Food preparation methods have a significant influence on HAA formation and much research has been devoted to identify the carcinogens in fried and broiled food. Marinating prior to cooking is one of preparation methods frequently used for meat products. However, there is not much information on the effect of commercial marinades on the formation of meat carcinogens, particularly heterocyclic aromatic amines. Thus, the objective of this study was to evaluate the commercial marinades for their ability to inhibit HAA formation and overall mutagenicity in fried beef steaks.

Materials and Methods

Safety All heterocyclic aromatic amines are mutagenic and/or carcinogenic; accordingly, all extractions, separations, and handling of pure compounds were performed with appropriate safety precautions, including the use of goggles, latex gloves, and efficient fume hoods.

Materials Commercial marinade sauces were obtained from CJ Corporation (Seoul, Korea). Dimethyl sulfoxide (DMSO) was purchased from Fluka Chemical Co. (Buchs, Switzerland) and 2-aminoanthracene was obtained from Sigma Chemical Company (St. Louis, MO, USA). The HAA standards (MeIQx, 4,8-DiMeIQx, and PhIP) were obtained from Toronto Research Chemicals (Toronto, Canada). The HAA standard (FEMA-Flavor and Extracts Manufacturer's Association) and the internal standard, caffeine, were kind gifts from Dr. Mark Knize, Lawrence Livermore National Laboratory (Livermore, CA, USA). The FEMA standard contained IQ, MeIQ, MeIQx,

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DiMeIQs, and PhIP, each at 5 ng/μL. Propyl-sulfonic acid (PRS) Bond-Elut columns (500 mg) and C18 (100 mg) cartridges were purchased from Varian Inc. (Harbor City, CA, USA). Extrelut-20 columns and Extrelut diatomaceous earth were obtained from E.M. Separations Technology (Gibbstown, NJ, USA). All other chemicals were of analytical grade and were purchased from Fisher Scientific (Fair Lawn, NJ, USA).

Fresh beef steaks were obtained from a local supermarket and used within 1 hr of purchase, or stored at -20°C until use.

Preparation of meat samples Three different commercial marinade sauces were evaluated to determine the effect of the each ingredient on HAA formation and overall mutagenicity in beef steaks. Marinade A consisted of high fructose corn syrup, Korean hot pepper paste, salt, soy sauce, garlic powder, pear puree, black pepper, citric acid, ginger extract, caramel, xanthan gum, and sesame. Marinade B consisted of the same ingredients of Marinade A, except for Korean hot pepper; whereas, Marinade C consisted of ingredients of Marinade B plus mushroom puree instead of pear puree. Beef steaks (8 cm × 8 cm, 1.5 cm in thickness; ~120 g) were marinated for 4 hr with 45 mL of each of the three different marinades at 4°C in Cleanwrap Zipper™ bags. Control samples (beef steak) were also placed in Cleanwrap Zipper™ bags with 45 mL of water and were treated identically to the test samples until required for cooking. Meat samples were fried in a teflon-coated electric frying pan (Chefline Corp., Seoul, Korea) at 225°C (surface temperature) for 10 min on each side. The surface temperature of the frying pan and the internal temperature of patties were determined using a surface temperature thermometer and a thermocouple thermometer (Pacific Transducer Corp., Los Angeles, CA, USA). Internal temperature of the patties at the end of frying (20 min) was 88±5°C. Two meat samples were fried for each replication, and three replicates were analyzed for each treatment. For each replicate, four subsamples were analyzed (two unspiked for concentration and two spiked for recovery). The cooked meat samples were mixed in a blender to produce a uniform sample and frozen at -4°C until extraction.

Extraction of HAAs from meat samples and HPLC analyses The HAAs were extracted from the meat samples and purified by solid-phase chromatography following the procedure of Gross and Grüter (16) (Fig. 1) Analysis of the HAAs was carried out on a TSK-gel ODS80-TM column (25 cm × 4.6 mm id; Tosoh Haas, Montgomeryville, PA, USA) following the procedure of Shin and Lee (16).

***Salmonella* mutagenicity assay** The mutagenic activity of the sample extracts was determined using the standard plate incorporation assay described by Ames *et al.* (17) using *Salmonella typhimurium* TA98 (Molecular Toxicology, Inc., Boone, NC, USA). Aroclor-induced rat liver S-9 mixture (Molecular Toxicology, Inc) was used for metabolic activation. DMSO was used as a negative control (spontaneous revertant colonies), while 2-aminoanthracene was used as a positive control for *S. typhimurium* TA98. The latter gave an average of 850 revertants/mg. Mutagenic activity was calculated from the linear portion of the dose-response curve using the method of Moore and Felton (18). A minimum of 4 dose points from duplicate platings were used, and the linear portion of the curves was used to calculate the revertants/g of cooked meats.

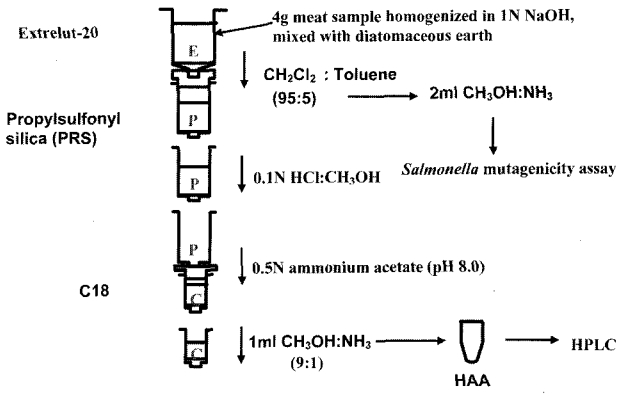

Fig. 1. Solid phase extraion scheme for the detection of HAAs and to determine their mutagenicity in food system.

Statistical analyses The results were analyzed by Sigma Stat 2.0 (Jandel Corp., San Rafael, CA, USA). One-way analysis of variance (ANOVA) was performed. Appropriate comparisons were made using the Student-Newman-Keuls test for one-way ANOVA analysis. Calculation of mutagenic activity was made by linear regression analysis of the dose response curves of revertants/g of meats.

Results and Discussion

Reduction of HAA formation in fried beef steaks The dominant HAA in fried beef steaks was PhIP, followed by MeIQx, and DiMeIQx (Table 1). IQ and MeIQ are found infrequently in cooked beef, and presence of very small concentrations of IQ and MeIQ compounds in cooked beef are analytically problematic because of difficulties with co-elution and peak interference (19). Average recoveries of HAAs added to the cooked pork chops, with or without marinades, were 78±13, 85±14, and 79±12% for MeIQx, DiMeIQx, and PhIP, respectively. These recoveries are comparable to those reported by Balogh *et al.* (8). Salmon *et al.* (20) reported recoveries ranging from 35 to 98% for IQ, MeIQx and DiMeIQx and from 9 to 63% for PhIP, while Shin *et al.* (9) reported average recovery percentages of 74±16, 74±19, and 66±16% for MeIQx, Di MeIQx, and PhIP, respectively.

Three different commercial marinades A, B, and C tested individually reduced the total HAA formation in fried beef steaks by 44, 38, and 40%, respectively after 4 hr of marinating time (Table 1). Higher inhibition of HAA formation was achieved with commercial marinade containing Korean hot pepper paste (A) than pear puree or mushroom puree (B and C). Korean hot pepper paste in commercial marinade A may be involved in causing increased levels of HAAs. Lee *et al.* (21) reported that the presence of capsaicin in red pepper exhibited antioxidant effect on lipid oxidation. It is anticipated that capsaicin

Table 1. Effect of three different commercial marinades on the formation of heterocyclic aromatic amines in fried beef steaks[1)]

Treatment	Heterocyclic aromatic amines (ng/g)[2,3)]				
	MeIQx	DiMeIQx	PhIP	Total HAAs	Inhibition (%) of total HAA formation
Control	5.5±0.5[a]	3.4±0.3[a]	17.7±1.5[a]	26.6	
Marinade A[a]	3.0±0.3[b]	1.7±0.3[b]	10.3±0.5[b]	15.0	44
Marinade B[b]	3.3±0.3[b]	2.0±0.3[b]	11.2.±1.4[b]	16.5	38
Marinade C[c]	3.2±0.4[b]	2.0±0.3[b]	10.9±1.6[b]	16.1	40

[1)]Values are based on measured cooking losses for individual meat samples.
[2)]Means with the same superscript are not significantly different ($p<0.05$)
[3)]Data are the means and standard deviations of three replicates.
[a]Marinade A consisted of high fructose corn syrup, Korean hot pepper paste, salt, soy sauce, garlic powder, pear puree, black pepper, citric acid, ginger extract, caramel, xanthan gum, and sesame.
[b]Marinade B consisted of high fructose corn syrup, salt, soy sauce, garlic powder, pear puree, black pepper, citric acid, ginger extract, caramel, xanthan gum, and sesame.
[c]Marinade C consisted of high fructose corn syrup, salt, soy sauce, garlic powder, mushroom puree, black pepper, citric acid, ginger extract, caramel, xanthan gum, and sesame.

compound with antioxidant activity are involved in bringing about higher percentage of inhibition on HAA formation. The mechanisms by which antioxidants inhibit mutagen formation have not been fully elucidated. One possible mechanism is that antioxidants function as free radical scavengers act in the early stages of the Maillard reaction prior to the Amadori rearrangement. There was, however, no significant difference in the inhibition achieved with three different commercial marinades. Salmon *el al.* (20) reported that marinating chicken breasts in a mixture of brown sugar, olive oil, cider vinegar, garlic, mustard, lemon juice, and salt for four hours prior to grilling decreased PhIP concentration by 92-99%. They concluded that marinating chicken coupled with twenty minutes or less of cooking time would prevent the formation of high levels of HAAs. However, our results are inconsistent with those reported by Nerurkar *et al.* (22) who demonstrated that beefsteaks marinated in commercial barbecue sauce containing honey, high-fructose corn syrup, vinegar, concentrated tomato juice, modified food starch, and salt increased PhIP and MeIQx formation by 1.9 and 2.9 folds. They also demonstrated that teriyaki sauce (16-20 h) and grilled for ten and fifteen minutes decreased MeIQx by 44% and 66%, respectively. Levels of PhIP also decreased by 45% and 67% under the same conditions. Similar decreases in MeIQx and PhIP were observed when beefsteaks were marinated in turmeric-garlic sauce.

Major reduction of HAA formation with commercial marinade sauces may due to the presence of carbohydrate compounds and organosulfur compounds of garlic in the sauce. Shin *et al.* (23) demonstrated that various sulfur compounds in garlic reduced the HAA formation in fried ground beef patties ranging from 65 to 78%. Shin *et al.* (24) reported that carbohydrate components are the primary inhibitory compounds in HAA formation in fried ground beef patties. They observed that addition of 0.39 g (w/w) fructose or 0.36 g glucose produced similar inhibition of HAA formation (24). It has been established that glucose has a great impact on the formation of HAAs and also changes the relative amounts of the HAAs. However, when excess amounts of carbohydrates are added to meat or model system containing HAA precursors, formation of HAAs is reduced (26-27). The mechanisms by which carbohydrates inhibit HAA formation have not been fully established. One possible explanation is that sugar itself, or more likely, some Maillard reaction products may combine directly with creatine or creatinine, and that such a reaction may be competitive with the reaction that produces HAAs. The Maillard reaction is facilitated by the addition of honey as the principal reactant of reducing sugar to the meat samples, which contain free amino group as a meat protein. Additional support was shown by decreased HAA formation and overall mutagenicity by Maillard reaction products. Lee *et al.* (25), and Jenq *et al.* (26) demonstrated that Maillard reaction products such as pyrazine, pyridines, thiophene, and thiazole compounds reduced the HAA formation and mutagenicity in model system study. They summarized that increasing the concentrations of Maillard reaction products by addition of sugar resulted in reduction in HAA formation and mutagenicity.

Reduction of overall mutagenicity in fried beef steaks

The mutagenic activity of control beef steaks (beef steaks only marinated with water) was 718 revertants/ g of meat. The reduction in the overall mutagenicity of fried beef steaks was achieved with three different commercial marinades (A, B, and C) by 47, 42, and 44% respectively, with the number of revertants being lowered from 718 revertants/g of meat to 384, 413, and 401 revertants/g of meat, respectively. There was, however, no significant difference in the percent reduction achieved by these three commercial marinades (Table 2).

Some of these results are comparable to those reported by Nerurkar *et al.* (22) who demonstrated a 41 to 47% reduction in overall mutagenicity in fried beefsteak by marinating with teriyaki sauce. They also observed that marinating the steaks with tumeric-garlic sauce resulted in 34% and 45% lower mutagenicity at 10 min and 15 min of cooking, respectively. Salmon *et al.* (20) observed a decrease in mutagenic activities of cooked chicken that reflected the effect of marinade on the levels of HAAs. However, they could not assess which component should be responsible for inhibition of HAAs because of the complex nature of the marinades.

The mutagenic activity of each of the HAA standard was determined in *S. typhimurium* TA 98 by Ames test. The mutagenic activity of MeIQx, DiMeIQx, and PhIP standard determined in the *S. typhimurium* TA 98 were 82,900, 19,800, and 1,900 revertants/μg of HAA, respec-

Table 2. Effect of three different commercial marinades on overall mutagenicity measured by *S. typhimurium* TA98 in fried beef steaks

Treatment	Revertants/g of meat[1,2)]	Reduction (%) of the overall mutagenicity
Control	718.0±23.5[a]	
Marinade A[a]	383.7±15.8[b]	47
Marinade B[b]	413.1±11.4[b]	42
Marinade C[c]	401.2±11.2[b]	44

[1)]Means with the same superscript are not significantly different ($p<0.05$).
[2)]Data are the means and standard deviations of three replicates.
[a]Marinade A consisted of high fructose corn syrup, Korean hot pepper paste, salt, soy sauce, garlic powder, pear puree, black pepper, citric acid, ginger extract, caramel, xanthan gum, and sesame.
[b]Marinade B consisted of high fructose corn syrup, salt, soy sauce, garlic powder, pear puree, black pepper, citric acid, ginger extract, caramel, xanthan gum, and sesame.
[c]Marinade C consisted of high fructose corn syrup, salt, soy sauce, garlic powder, mushroom puree, black pepper, citric acid, ginger extract, caramel, xanthan gum, and sesame.

tively. These results are in agreement with the data of Felton and Knize (27) who reported the mutagenic activity of each HAA standard (MeIQx = 99,000 revertants/μg; DiMeIQx = 32,000 revertants/mg; PhIP = 2,000 revertants/μg). Although PhIP contributes less than 18% of the total mutagenic activity of meat, it is the most abundant HAA formed in cooked meat.

We were interested in correlating the measured mutagenicity of the fried patties by the *S. typhimurium* assay with mutagenicity values calculated from the concentrations of the HAAs in the fried ground beef that were quantified by HPLC. This analysis is a guide to the amount of mutagenic activity not totally accounted for by HAA concentrations we measured. The plot of measured and calculated mutagenicity is shown in Fig. 2. The measured mutagenicity in each sample was similar (slope 1.13, R^2 = 0.94) to the mutagenicity values calculated from the determined concentrations of HAAs, but not all, of the mutagenicity detected. The scatter in the data is probably a combination of measurement errors in multistep solid-phase extraction procedures and the

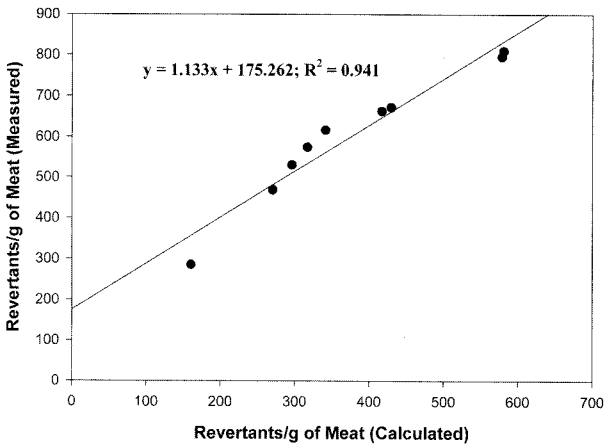

Fig. 2. Plot of mutagenic activity measured in S. typhimurium TA98 and mutagenic activity calculated from the amount of heterocyclic aromatic amine measured in fried beef steaks using the HPLC.

accumulation of errors in each analytical method. Turesky *et al.* (28) reported that about 80% of the mutagenicity could be accounted for by quantitative HPLC analyses of HAAs in cooked beef. On the basis of the results of the present study, *S. typhimurium* mutagenicity assay would be a reasonable screening method to determine HAA formation in cooked meat samples. In conclusion, this study demonstrates that commercial marinades may represent an effective approach to reduce HAA formation in fried beef steaks. Reduction of overall mutagenicity was related to the decrease in HAA formation in fried beef steaks. These observations should be of interest to the food processing industry, to restaurateurs, and to homemakers as a simple, practical method of achieving significant reductions of specific HAAs and of total mutagens in fried meat. Further research is necessary to study the better understanding of mechanisms by which honey components inhibit HAA formation.

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