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Influence of Genotoxic Heterocyclic Aromatic Amine Formation and Overall Mutagenicity in Ground Beef Patties Using Korean Bramble (*Rubus coreanum* Miquel)

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Abstract The effects of temperature and Korean bramble (*Rubus coreanum* Miquel) tissue concentrate on heterocyclic aromatic amine (HAA) formation in fried ground beef patties were investigated. Various amounts of Korean bramble tissue (4.0, 7.0, and 11.0%, w/w) were added to ground beef patties were fried at 2 different temperatures (190 and 225°C) for 10 min/side. It was observed in the fried ground beef patties fried at 190°C with the addition of 11.0%(w/w) Korean bramble that the mutagenicity decreased by 64%, and formation of 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]-pyridine(PhIP) reduced by 55 and 86%, respectively. Although no difference in total mutagenicity was shown in patties fried at 225°C with the addition of 4.0, 7.0, and 11.0%(w/w), different levels of reduction of PhIP formation in patties fried at 225°C with the addition of 4.0, 7.0, and 11.0%(w/w) were shown 49, 63, and 75%, respectively.

Keywords: heterocyclic aromatic amine, Korean bramble, mutagenicity, ground beef

Introduction

Epidemiological studies have shown that diet and life style are closely related to human cancer. Many mutagens and carcinogens have been identified in foods. Recently, several foods and constituents of foods have been investigated for their inhibitory or promotional effects on carcinogenesis (1, 2).

A group of mutagenic and/or carcinogenic heterocyclic aromatic amines (HAAs) has been found on the charred surfaces of meat and fish. The most common heterocyclic aromatic amines identified in fried ground beef are: 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-trimethylimidazo[4,5-*f*]quinoxaline), and PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-*b*]-pyridine). Other HAAs have been isolated and identified in meat and model systems; altogether, this group comprises 16 mutagenic HAAs and 2 comutagenic HAAs (3).

The daily intake of MeIQx and PhIP is estimated to be 0.5-1.8 and 0.1-13.8 μg, respectively. Heterocyclic aromatic amines are mutagenic in the Ames Assay, with specific activities of IQ, MeIQ, MeIQx, DiMeIQx, and PhIP ranging from 120 to 661,000 revertants/μg (4). 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 (5).

It has been reported that concentrations of HAAs or overall mutagenicity in fried ground beef patties can be reduced by addition of compounds, such as oligosaccharides (6), vitamin E (7), garlic related sulfur compounds (8, 9) soy protein concentrate (10), defatted glandless cottonseed flour (11), and tea polyphenolics (12). Deighton *et al.* (13) found that Korean bramble had the antioxidant capacities ranged from 0.03 to 7.98 μmol Trolox equivalents 1/g (TEAC) or from 191 to 13078 μmol/L ferric reducing antioxidant power (FRAP). The addition of plant tissue (11.5%, w/w) to ground beef patties prior to cooking resulted in a substantial reduction of HAA formation. Specifically, IQ, MeIQ, MeIQx, DiMeIQx, and PhIP were decreased 72.1, 50, 62, 81, and 92.7%, respectively. However, the relationship of the Korean bramble tissue concentrate to HAA inhibition is unknown.

Therefore, the objectives of this study were to investigate the formation of HAAs in fried ground beef patties containing Korean bramble cooked at 2 different temperatures, to establish a dose-response relationship of added Korean bramble tissue to ground beef patties, and to determine overall mutagenicity of these ground beef patties.

Materials and Methods

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

Reagents The HAA standards (MeIQx, 4, 8-DiMeIQx, and PhIP) were purchased from Toronto Research Chemicals (Toronto, Canada). The HAA standard (FEMA - Flavor and Extracts Manufacturer's Association) were gifts from Dr. Mark Knize, Lawrence Livermore National laboratory, Livermore, CA, USA. The FEMA standard contained IQ, MeIQ, MeIQx, DiMeIQs, and PhIP, each at 5 ng/μL. A polysulfonic acid (PRS) Bond Elute columns

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(500 mg), C-18 cartridges (100 mg), and hydromatrix were purchased from Varian, Inc. (Harbor City, CA, USA). Extrelut– 20 columns were obtained from E. M. Separations Technology (Gibbstown, NJ, USA). All other chemicals were of analytical grade and were obtained from Fisher Scientific (Fair Lawn, NJ, USA).

Materials Fresh ground beef was obtained from a local supermarket and Korean brambles were obtained from were obtained from Bokbunja Farm (Gochang, Jeonnam, Korea). The Korean brambles were flushed with nitrogen in freezer bags prior to their storage at -20°C until ready to use. The fat content of the meat (14±2%) was determined using the method of Folch *et al.* (14).

Preparation of ground beef patties Patties were prepared from ground beef (14±2% fat) as follows: control patties (no Korean bramble tissue added) and patties containing 3 levels of Korean bramble tissue (4.0, 7.0, and 11.0%, w/w). Patties were prepared by mixing the pulverized Korean bramble tissue with the ground beef in a mixer. Patties were formed by weighing 100 g of meat in a petri dish (9×1.5 cm) to ensure patty uniformity. Patties were then frozen and stored at -20°C until ready to use.

Cooking of patties Beefsteaks were fried in a teflon-coated electric frying pan (Chefline Corp., Seoul, Korea) at 190 and 225°C (surface temperature) for 10 min 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±4°C. Two steaks were fried for each replication, and 3 replicates were analyzed for each treatment. For each replicate, 4 subsamples were analyzed (2 unspiked for concentration and 2 spiked for recovery). The cooked beefsteaks were mixed in a blender to produce a uniform sample and frozen at -4°C until extraction.

Extraction of HAAs from meat samples for HPLC analyses and mutagenic activity testing The HAAs were extracted from the meat samples and purified using solid-phase chromatography following the procedure of Gross and Grüter (15). Separation of the HAAs was carried out on a TSK-gel ODS80-TM column (25 cm × 4.6 mm i.d.; Tosoh Haas, Montgomeryville, PA, USA) following the procedure of Shin and Lee (16).

Mutagenicity assay with *Salmonella* 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. Dimethyl sulfoxide (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.

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

The recoveries of HAAs present in the cooked ground beef with and without addition of the Korean bramble tissue ranged from 31 to 76%. The average recoveries of spiked samples were 50±6, 37±4, and 49±5% for MeIQx, DiMeIQx, and PhIP, respectively. These results are comparable to those of other workers who reported similar recoveries ranging from 32 to 98% (19, 20).

As shown in Table 1, MeIQx was more abundant and the yield of MeIQx was in a total of 7.86±1.3 ng/g of meat in the control. Addition of the 11% Korean bramble tissue to ground beef decreased the formation of MeIQx by 55% ($p<0.05$). Lower levels of the Korean bramble tissue in ground beef did not significantly inhibit at 190°C. A significant reduction in the formation of PhIP was found at all the levels (4.0, 7.0, and 11.0%, w/w), which inhibit of 32.1, 71.7, and 86.3%, respectively. As expected, the higher temperature produced a greater quantity of HAAs. These results were consistent with the previous study by Arvidsson *et al.* (21) which showed that MeIQx formed at the highest rates at 225°C followed by 7,8-DiMeIQx and 4,8-DiMeIQx. Adding the Korean bramble tissue (4.0, 7.0, and 11.0%, w/w) to ground beef patties significantly reduced the formation of PhIP ranged from 48.9 to 75.1% (Table 2). MeIQx and DiMeIQx did not dramatically change in the addition of the Korean bramble tissue. However, formation MeIQx tended to decrease whereas formation DiMeIQx tended to increase in addition of the Korean bramble tissue.

The cooking temperature plays a key role in the formation of HAAs and their amount. Skog *et al.* (22) fried an array of meat products at temperatures ranging from 150 to 225°C and reported the highest levels of HAA

Table 1. Effect of Korean brambles tissue addition on the formation of heterocyclic aromatic amine in ground beef patties fried at 190°C[1)]

Sample	MeIQx (ng/g)	% Inhibition	PhIP (ng/g)	% Inhibition
Control	7.86±1.3[a]		4.24±1.2[a]	
4.0%	10.42±2.1[a]	NSD	2.88±1.0[b]	32.1
7.0%	6.26±1.8[a]	NSD	1.20±0.4[b]	71.7
11.0%	3.54±1.2[b]	55.0	0.58±0.2[b]	86.3

[1)]Values are expressed on a cooked ground beef basis; Data represent the mean±SD of 5 analyses per treatment (n=5). Means in the same columns bearing different superscripts are significantly different ($p<0.05$). NSD= No significant difference.

formation were found at the highest temperatures. Our results demonstrated similar trends in the ground beef patties at a lower temperature (190°C) produced 2 kinds of HAAs at a lower levels, while patties at a higher temperature (225°C) produced 3 kinds of HAAs significantly at higher levels.

Although, our results clearly show decreases in formation of major HAA species, it is also important to independently establish reduction of overall mutagenicity of the cooked product. It is also possible that the presence of the Korean bramble tissue could lead to an increase in the formation of other mutagenic compounds. Therefore, the mutagenic activities of cooked ground beef patties with and without the Korean bramble tissue were evaluated by the Ames assay. *S. typhimurium* TA98, was used because HAAs are more likely to induce a frameshift mutation than a basepair mutation (4). Ground beef patties fried at 190°C in the addition of the Korean bramble tissue at the 7.0 and 11.0% treatment levels reduced mutagenicity by inhibition of 41.5 and 64.3%, respectively (Table 3). These findings were consistent with the inhibition levels of individual HAAs presented in Table 1. The ground beef patties fried at 225°C showed a trend toward decreased mutagenic activity, but were not significant. Although the level of PhIP formation was inhibited by as much as 75.1% in the 11.0% treatment level, the amount of DiMeIQx tended to increase. Since DiMeIQx is over 100 times more mutagenic than PhIP, even a 7-fold reduction in PhIP (Table 2) is likely masked by small changes in DiMeIQx. Therefore, mutagenicity results are consistent with HAA levels measured. The addition of antioxidants to meat prior to cooking has shown to be effective at inhibiting the formation of HAAs. Kato *et al.* (23) suggested that phenolic antioxidants effectively scavenge the unstable free radical Maillard intermediates, thus preventing the formation of HAAs. Weisburger *et al.* (24) showed inhibition of PhIP in model systems containing glucose, creatinine, and phenylalanine using green and black tea and individual polyphenols derived from tea. These antioxidants showed a significant decrease in mutagenic activity ranging from 29 to 91%. Our results of addition of the Korean bramble tissue to ground beef provide another means of inhibiting the formation of HAAs. It may be possible that antioxidants present in the Korean bramble tissue are responsible for inhibiting HAA formation.

This study clearly demonstrated a dose-response inhibition associated with addition of Korean bramble tissue to ground beef patties. The addition of the Korean bramble tissue at 11.0% yielded the greatest inhibitory effect and the least mutagenic activity. The amounts of HAAs formed at a higher frying temperature were significantly greater than those formed at a lower frying temperature. Further research is in progress to better understand which compound or compounds present in the Korean bramble tissue are responsible for inhibition of HAA formation.

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Table 2. Effect of Korean brambles tissue addition on the formation of heterocyclic aromatic amine in ground beef patties fried at 225°C[1)]

Sample	MeIQx (ng/g)	Inhibition (%)	DiMeIQx (ng/g)	Inhibition (%)	PhIP (ng/g)	Inhibition (%)
Control	14.16±4.0[a]	-	4.83±2.0[a]	-	16.48±3.0[a]	-
4.0%	16.15±4.0[a]	NSD	6.21±3.4[a]	NSD	8.42±2.3[b]	48.9
7.0%	13.77±3.7[a]	NSD	7.34±1.9[a]	NSD	6.11±1.4[b]	62.9
11.0%	9.47±2.1[a]	NSD	9.31±3.1[a]	NSD	4.10±0.4[b]	75.1

[1)]Values are expressed on a cooked ground beef basis. Means in the same columns bearing different superscripts are significantly different ($p<0.05$). Data represent the mean ±SD of 5 analyses per treatment (n=5). NSD = No significant difference.

Table 3. Mutagenic activities of ground beef patties with differing Korean brambles content fried at 190 and 225°C[1)]

Sample	Frying temp. 190°C mutagenicity (revertants/g of meat)	Inhibition (%)	Frying temp. 225°C mutagenicity (revetants/g of meat)	Inhibition (%)
4.0%	1087±112[a]	NSD	1421±111[a]	NSD
7.0%	664±134[b]	41.5	1286±121[a]	NSD
11.0%	405±78[b]	64.3	1301±141[a]	NSD

[1)]Data represent the mean±SD of 3 analyses per treatment (n=3). Means in the same columns bearing different superscripts are significantly different ($p<0.05$). NSD, no significant difference.

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