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Influence of Food Ingredients on the Formation of Heterocyclic Aromatic Amine in Cooked Pork Patties

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Abstract The effects of cooking method, cooking time and various food ingredients on the formation/ inhibition of heterocyclic aromatic amines (HAAs) in pork products were investigated. Three HAAs, 2-amino-3,8-dimethylimidazo [4,5-f] quinoxaline ($MeIQ_X$), 2-amino-3,4,8-trimethylimidazo [4,5-f] quinoxaline ($DiMeIQ_X$) and 2-amino-1-methyl-6-phenylimidazo [4,5-b] pyridine (PhIP) were measured in pork products using solid-phase extraction and HPLC. Pork patties were boiled, oven-broiled and pan-fried to internal temperatures of 71, 77 and 88°C. Generally, HAA concentrations increased with increasing internal temperature, and HAA formation was greatest with pan-fried. Selected food ingredients (vitamin E, sodium nitrite, sodium tripolyphosphate, sodium ascorbate, Nanking cherry tissue and cherry tissue extract) inhibited HAA formation in pork patties fried at 225 °C for 10 min/side, with the greater inhibition provided by cherry tissue and its methanolic extract.

Key words: Heterocyclic aromatic amine, food ingredients, genotoxic, pork product

Introduction

Some human epidemiological studies have shown that a high consumption of well-done, red meat is associated with an increased risk of developing cancer. It is important to quantify dietary exposures to understand an individual's risk for cancer and to identify habits that change an individual's risk. The daily intake of $MeIQ_X$ (2-amino-3,8-dimethylimidazo[4,5-*f*]-quinoxaline) and PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine) is estimated to be 0.5~1.8 and 0.1~13.8 μg, respectively (1). Heterocyclic aromatic amines (HAAs) are mutagenic in the Ames Assay using *Salmonella typhimurium* TA98, with specific activities of IQ (2-amino-3-methylimidazo[4,5-*f*]-quinoline), MeIQ (2-amino-3,4-dimethylimidazo[4,5-*f*]-quinoline), MeIQx, DiMeIQx (2-amino-3,4,8-trimethylimidazo[4,5-*f*]-quinoxaline), and PhIP 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 thought to be 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 the 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 the addition of reducing sugars to meat beyond the optimum needed for formation of HAAs results in the formation of Maillard reaction products which inhibit the mechanism of HAA formation (14). Pork is one of the most frequently consumed meats in Korea and could contribute to the HAA exposure of the general population. Food ingredients have a significant influence on HAA formation and much research has been devoted to the carcinogens in cooked food. The objectives of this study were to evaluate the effect of three common cooking procedures (boiling, oven-broiling, pan-frying) on HAA formation in fried pork patties, and to investigate the influence of selected food ingredients on HAA formation in ground pork patties.

Materials and Methods

Safety All HAAs are mutagenic and/or carcinogenic. Therefore, 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 The HAA standards (MeIQx, 4,8-DiMeIQx, and PhIP) were obtained from Toronto Research Chemicals (Toronto, Canada). The HAA standard (FEMA-Flavor and the Extracts Manufacturer's Association) and the internal standard, caffeine, were gifts from Dr. Mark Knize, Lawrence Livermore National Laboratory (Livermore, CA, USA). The FEMA standard contained IQ, MeIQ, MeIQx, DiMeIQx, 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, U.S.A.). Extrelut-20 columns and

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Extrelut diatomaceous earth were obtained from E.M. Separations Technology (Gibbstown, NJ, USA). Vitamin E (α-tocopherol), sodium ascorbate, sodium nitrite and sodium tripolyphosphate were purchased from Sigma Chemical Company (St. Louis, MO, USA). All other chemicals were of analytical grade and were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Freshly ground pork and pork chops were purchased from a local supermarket and used within 1hr of purchase or stored at -20°C until required. Frozen, pitted Nanking cherries were obtained from Yujung Farm (Daejeon, Korea). The Nanking cherries were flushed with nitrogen in freezer bags prior to storage at -20°C.

Cooking of pork patties The ground pork had a fat content of 21% as determined by the method of Folch *et al.* (15). Patties weighing 100 g were formed in a petri dish (9cm diameter x 1.5 cm thickness) to ensure patty uniformity, vacuum packed and stored at -20°C until use. The patties were cooked by three methods. In *boiling*, the patties were put in Zip-lock™ bags and cooked in a boiling water bath to internal temperatures of 71 and 77 °C. In *oven-broiling*, the oven temperature was set at 177 °C and the patties were set ~15 cm away from the heating element and cooked to internal temperatures of 71 and 77 °C. In *pan frying*, a teflon-coated electric frying pan was set at 177°C and the patties were fried to internal temperatures of 71 and 77°C. The internal temperatures of the patties were monitored by a thermocouple (Pacific Transducer Co., Los Angeles, CA).

To further enhance HAA formation, additional studies were performed using a higher cooking temperature. For oven-broiling and pan-frying, the cooking temperature was set at 225°C and the patties were cooked to an internal temperature of 88°C. Cooking parameters for both studies are summarized in the Table 1. Three experimental replications were performed and four sub-samples of each replication were analyzed for HAA content.

Addition of potential HAA inhibitors to pork patties Ground pork with 15% fat was used to prepare 100 g patties. Vitamin E (1% based on fat content) was dissolved in 1 ml corn oil and added to the ground pork before formulating into the patty. Patties containing sodium nitrite (150 ppm), sodium tripolyphosphate (0.35%) and sodium ascorbate (0.5%) were prepared by dissolving the ingredients in 1 mL water and adding directly to the ground pork. Patties containing 11.5% tart cherry tissue or a methanolic extract of ground cherry tissue were prepared as described by Britt *et al.* (16). The patties were allowed to stand for 2 hrs before frying. Control patties contained either 1 ml corn oil or 1 ml water which was added to the ground pork. For each replication, two ground pork patties were pan fried at 225°C for 10 min/side, giving a total cooking time of 20 min. Three experimental replications were performed and four sub-samples of each replication were analyzed for HAA content.

Extraction of HAAs from meat samples and HPLC analyses HAAs were extracted from the meat samples and purified using solid-phase chromatography following the procedure of Gross and Grüter (17) (Fig. 1). HAAs were separated 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 (18).

Statistical analyses The results were analyzed by one-way analysis of variance (ANOVA) using Sigma Stat 2.0 (Jandel Corp., San Rafael, CA, USA). Appropriate comparisons were made using the Student-Newman-Keuls test for one-way ANOVA analysis.

Results and Discussion

Effect of cooking conditions on HAA formation in ground pork patties Ground pork patties were cooked using the three common household cooking procedures (boiling, broiling and frying) to internal temperatures of 71 °C, 77°C and 88°C. The dominant HAA in the cooked pork patties was PhIP, followed by MeIQx, and DiMeIQx (Fig. 2). Pan-frying had the greatest effect on HAA formation, followed by broiling and boiling. The boiled patties had the lowest HAA concentration at all cooking internal temperatures and cooking times. The HAA concentrations of the broiled patties peaked at an internal temperature of 71°C or 77°C. In general, total HAA formation increased with higher internal temperatures. However, increasing the internal temperature of the boiled

Table 1. Time and temperature parameters for the formation of heterocyclic aromatic amines in cooked pork patties[1]

Cooking method	Cooking temperature (°C)	Internal temperature (°C)	Cooking time (min)
Boiling	100	71	8
	100	77	10
	100	88	16
Broiling	177	71	12
	177	77	12
	225	88	19
Pan-frying	177	71	9
	177	77	11
	225	88	21

[1]Data are the means of three replicates.

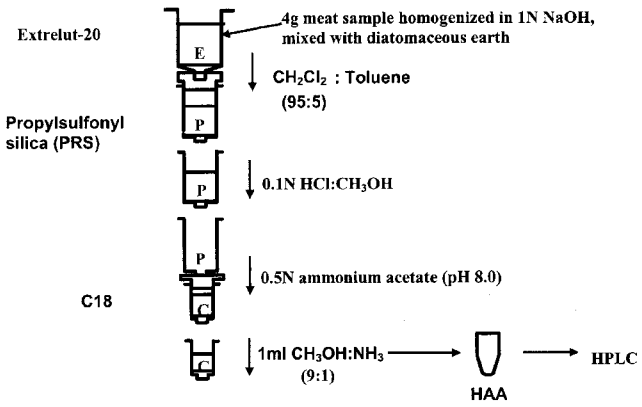

Fig. 1. Solid phase extraction to detect HAAs in the food system.

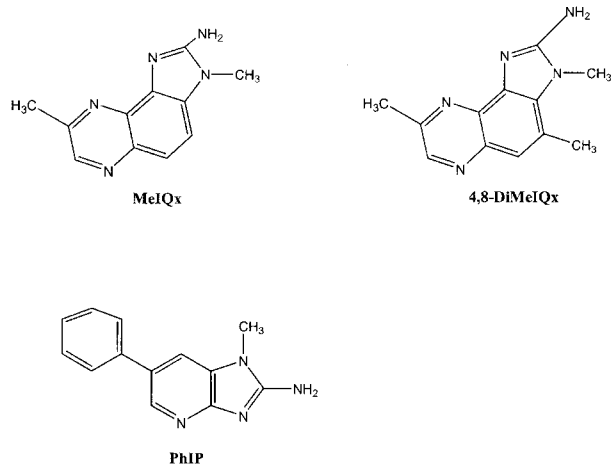

Fig. 2. Chemical structure of HAAs quantitated in cooked pork chops.

pork patties from 71 to 88 °C had no significant effect ($p>0.05$) on total HAA formation. When the patties were cooked to an internal cooking temperature of 71 or 77°C, there was no significant ($p>0.05$) difference among the three cooking methods and the broiled patties had the highest HAA formation. On the other hand, when the patties were cooked to an internal temperature of 88 °C, total HAA concentrations in the pan-fried patties were significantly ($p<0.05$) greater than those in the broiled and boiled patties. PhIP level was not detected in the boiled patties, nor in the broiled patties cooked to 71 and 77°C. However, PhIP concentration was 2.7 ng/g in the boiled patties cooked to 88°C. On the other hand, PhIP concentrations increased from 0.3 to 10.5 ng/g in pan-fried patties when the temperature increased from 71 to 88°C, respectively (Table 2). These observations agree with

Table 2. Effect of different cooking methods at three cooking internal temperatures on the formation of heterocyclic aromatic amines in cooked pork patties[1]

	Heterocyclic aromatic amines (ng/g)[1,2]			
Treatment	MeIQx	DiMeIQx	PhIP	Total HAAs
Boiled				
71°C	0.4±0.1[a]	nd	nd	0.4
77°C	0.8±0.2[a]	nd	nd	0.8
88°C	1.0±0.2[b]	nd	nd	1.0
Broiled				
71°C	1.2±0.3[b]	0.3±0.2[a]	nd	1.5
77°C	1.4±0.6[b]	0.5±0.3[a]	nd	1.9
88°C	1.6±0.5[b]	0.7±0.4[a]	2.7±0.4[b]	5.0
Pan-fried				
71°C	0.6±0.2[a]	0.3±0.1[a]	0.3±0.1[a]	1.2
77°C	0.9±0.3[a]	0.4±0.1[a]	0.5±0.2[a]	1.8
88°C	5.0±0.8[c]	1.7±0.7[a]	10.5±0.8[c]	17.2

[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.
Nd is not detected.

Knize *et al.* (5) who reported that the amount of PhIP is generally higher than that of MeIQx after longer cooking time. Skog *et al.* (19) reported that oven-roasting produces fewer HAAs than pan-frying, due to the less efficient heat transfer in air than when the product is in direct contact with the frying pan. When chicken and beef burgers were cooked in a convection oven, less mutagenic activity was formed in the presence of steam, which affected the heat transport and decreased the surface temperature of the products. Adamson (20) reported that oven roasting and baking, which cook mainly by heat convection, produced low to intermediate levels of mutagenicity in meat. Comparatively greater amounts of HAAs were detected in the fried and grilled samples. The physical contact of meat with a hot metal surface and direct contact with a flame appear to be responsible for these observations. Sinha *et al.* (21) also concluded that these factors are responsible for the greater concentrations of HAAs in flame-broiled (grilled) and pan-fried meat products. Smaller concentrations of HAAs were detected in cooked pork chops than those found in the pork patties cooked to a similar internal temperature. The comparatively smaller HAA concentrations in pork chops may be due to the integrity of the meat, resistance to leaking of precursors to the surface where they are exposed to higher temperatures and the uneven distribution of fat in pork muscle.

Inhibition of HAA formation in pork patties Pork patties were fried at 225°C for 10 min/side (total of 20 min) to generate sufficient HAAs to effectively evaluate the ability of selected food ingredients to inhibit their formation. MeIQx and PhIP were detected in both the control patties and those containing the added ingredients. Control patties had the greatest HAAs formation. The inhibitory effects of cherry tissue and Nanking cherry extract, sodium ascorbate, sodium nitrite, sodium triphosphate and vitamin E on total HAA formation in the cooked pork patties were varied. ANOVA analysis revealed that the addition of each ingredient had a significant effect ($p<0.05$) on total HAA reduction. Nanking cherry was the most effective inhibitor while sodium triphosphate was the least effective. Nanking cherry added to ground pork at the 11.5% level reduced total HAA concentrations by 88.6% for Nanking cherry tissue and by 90.7% for Nanking cherry extract. The addition of sodium ascorbate, vitamin E, sodium nitrite and sodium tripolyphosphate reduced total HAA formation by 67.9%, 55.8%, 46.7% and 32%, respectively. Table 3 shows the percentage of the amounts of MeIQx, DiMeIQx and PhIP in the cooked patties with each food ingredient, relative to control level. Nanking cherry tissue and its methanolic extract offered the greatest inhibition for PhIP formation, followed by DiMeIQx, and MeIQx. There was no significant difference ($p<0.05$) between Nanking cherry tissue and its methanolic extract on HAAs formation. The effective inhibition of HAA formation by Nanking cherry tissue agrees with the observation of Britt *et al.* (16) that ground beef patties containing Montmorency and Balaton cherry tissue significantly inhibited HAA formation when fried at 225 °C for 10 min per side. The basis of the inhibitory action of the cherry tissue has not been fully clarified.

When Vitamin E (1% of fat content) was added to

Table 3. Percent inhibition of different food ingredients on the formation of heterocyclic aromatic amines in fried pork patties

Food ingredient	Percent Inhibition of HAAs (%)[1]			
	MeIQx	DiMeIQx	PhIP	Total HAAs
Cherry tissue extract	53±7	57±18	96±4	91
Cherry tissue	56±4	71±16	93±6	87
Sodium ascorbate	32±5	0	73±5	68
Vitamin E	55±6	57±16	56±5	56
Sodium nitrite	51±5	14±4	47±6	47
Sodium triphosphate	43±4	0	32±4	32

[1]Data are the means of three replicates.

ground pork, a significant reduction (>50%) was observed on PhIP, DiMeIQx and MeIQx formation compared to the control patties. The inhibitory effect of vitamin E on PhIP formation in meat products has also been reported by Balogh *et al.* (8). Again, the mechanism of inhibition has not been fully evaluated, although the authors speculated that it could be limited to the antioxidant nature of vitamin E. When sodium nitrite (150 ppm) was added to ground pork, a significant reduction ($p<0.05$) was observed for PhIP and MeIQx, but a lower reduction for DiMeIQx. When sodium ascorbate (0.5%) was added to ground pork, PhIP was significantly reduced by 73% compared to MeIQx, which had less reduction. On the other hand, sodium triphosphate (0.35%) had the least inhibitory effect on PhIP and MeIQx. Both sodium ascorbate and sodium triphosphate had no effect on DiMeIQx formation.

The question remains of how these phenolic antioxidants minimize HAA formation in the fried ground pork patties. The mechanism(s) by which antioxidants inhibit mutagen formation has (have) not been fully elucidated. More studies are needed to further address the inhibition of HAA formation in fried meats by other natural antioxidants because HAAs are extremely mutagenic/carcinogenic. This will clearly involve more detailed studies on the mechanism of HAA formation in meat systems.

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