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S. Hinojosa Verdin

K.A. Hachmeister

J. Scott Smith

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## INHIBITION OF HETEROCYCLIC AMINE FORMATION IN BEEF PATTIES WITH ADDED SPICES AND INGREDIENTS

*S. Hinojosa Verdin, K. A. Hachmeister, and J. S. Smith*

### Summary

Heterocyclic amines (HCAs) are compounds present at part per billion levels in fried, grilled, broiled, barbecued and smoked meats. Most of these compounds are highly mutagenic, as demonstrated by the Ames test using *Salmonella typhimurium*. They also are carcinogenic in rodents and non-human primates following high dosage and long term oral administration. For decades, researchers have focused on inhibiting the production of these carcinogens. This research investigates the effects of natural antioxidants in spices or other ingredients on the reduction of heterocyclic amines formation when beef patties are cooked. The term "spice" in this paper includes herbs. Ground beef patties combined with different levels of added spices or ingredients were cooked at 375°F (5 minutes each side) or 400°F (7.5 minutes each side). Extracted HCAs were then analyzed using reversed-phase High Performance Liquid Chromatography (HPLC) with UV-Visible and fluorescence detectors. Of the spices used, basil added at 0.5% was most effective in decreasing HCAs. Of all the ingredients, food starches showed the best inhibition when added at 5% as they reduced MelQx, harman, and norharman forms of HCA at both 375°F and 400°F.

### Introduction

In 1977, extremely high mutagenic activity was reported in grilled beef and fish, which led to the discovery of the heterocyclic amines (HCAs). Further studies found that HCAs

were produced in meat and other protein rich products. Recently, MelQx, IQ, MelQ and PhIP, four of the most dangerous HCAs, were nominated by the National Toxicology Program to be included on the 10th and 11th Reports of Carcinogens. HCAs have been related to colon, breast, and prostate cancer in numerous studies.

HCAs can be divided into two groups: the amino-carbolines or pyrolysis products, and the amino-imidazo-azaarenes (AIAS). The amino-carboline HCAs are formed by the pyrolysis of amino acids and proteins, but the exact mechanism is still unknown. Their formation is not dependent on creatin(in)e precursors, and therefore they may be present in foods of vegetative origin. The AIAS are formed at ordinary cooking temperatures. These compounds are extremely mutagenic compared to the amino-carbolines and are more commonly and easily formed during cooking.

The levels of HCAs can be reduced by preventing the Maillard browning reaction intermediates from reacting with creatinine. The use of antioxidants as free radical scavengers have stabilized intermediates from the Maillard browning reaction, therefore reducing the HCAs.

Some naturally occurring antioxidants found in spices can decrease levels of HCAs. Mustard and green tea have decreased the level of MelQx in beef. Rosemary, thyme, sage, garlic, and cherry tissue have decreased MelQx, IQ, MelQ, and PhIP when applied on the surface of beef steaks. Until now, no stud-

ies have assessed the effect of these spices when formulated into beef patties prior to cooking. This study measured inhibition of heterocyclic amines by adding certain spices or other ingredients to ground beef.

### Experimental Procedures

Fine ground (1/8-inch plate) beef (20% fat) from the Meat Lab at Kansas State University was mixed manually with spices or other ingredients in batches of 25 lb for every level of added spice. Spice levels were 0.5 and 5% and ingredient levels were 2.5 and 5%. The spices (and herbs) included basil, garlic, ginger, onion, oregano, rosemary, sage, thyme, and turmeric. Dried plums were also included at 3 and 10%. Other ingredients included modified food starches (B990 and Firmtex), corn syrup solids (M200), rolled oat blend, whey protein isolate, whey protein concentrate, soy protein isolate, and soy protein concentrate. Patties were formed using patty molds to dimensions of 0.6 inch thick and 4 inches in diameter and weighing from 3.7 to 4.0 ounces. The 10 patties formed per level of spice were crust frozen on aluminum trays for 20 minutes at  $-40^{\circ}\text{F}$  to facilitate handling, then packaged. Control samples without spices were prepared in a similar manner. Samples were stored at  $0^{\circ}\text{F}$  prior to cooking.

The samples were cooked on a Teflon<sup>®</sup> covered electric grill with temperature controller (Toastmaster, Denver, CO). A temperature profile of the grill surface was obtained prior to cooking in order to validate that the patties were all cooked under the same conditions. Three patties of each added level of spices or ingredients were cooked at the same time for each temperature. Two temperatures were used:  $375^{\circ}\text{F}$  (5 minutes each side), and  $400^{\circ}\text{F}$  (7.5 minutes each side). The griddle was cleaned between spices or ingredients to avoid interferences. After cooking, the patties were

cooled to room temperature, then ground completely using a Micro-Mill.

All ground patties were stored at  $0^{\circ}\text{F}$  prior to extraction with 9 to 1 methanol:ammonium hydroxide and subsequent analysis. The extract was dried under pressure with  $\text{N}_2$  and suspended with 25  $\mu\text{l}$  of methanol. Eight  $\mu\text{l}$  of the sample was injected using a 20  $\mu\text{l}$  loop into a Hewlett-Packard 1090 A, series II HPLC (Palo Alto, CA) fitted with a photodiode array UV-visible detector and a programmable fluorescence detector. Data were then analyzed using an HP ChemStation. Separation was achieved with a silica-based reversed-phase TSK-Gel ODS-80TM column (25 cm  $\times$  4.6 cm, 5  $\mu\text{m}$ , 80  $\text{\AA}$ ; Toso Haas, Montgomeryville, PA). The mobile phase consisted of three solvents: solvent A, 0.01 M triethylamine (pH 3.2); solvent B, 0.01 M triethylamine (pH 3.6); solvent C, acetonitrile. The gradient profile was linear, and the program was 0-10 minutes, 5-15% C in A; 10-10.1 minutes, exchange of A with B; 10.1-10 minutes, 15-25% C in B; 20-30 minutes, 25-55% C in B, followed by 15 minutes for column equilibration.

The standards for polar HCAs (MeIQ and PhIP) and nonpolar HCAs (harman and norharman) were obtained from Toronto Research Chemicals (Toronto, Canada).

### Results and Discussion

Recovery of compounds calculated by the spiking of samples was 59.5% for MeIQx, 73.1% for PhIP, 51.0% for norharman, and 43% for harman. Of the spices used, basil added at 0.5% was most effective in decreasing HCAs. At 0.5%, basil decreased MeIQx and PhIP, while having no effect ( $P>0.05$ ) on the co-mutagenics harman and norharman. Onion did not inhibit HCAs, but increased norharman and MeIQx when used at 5% in

patties cooked at 400°F, and increased harman and PhIP at both levels of addition. Dried plums were not very effective either, as they increased MelQx and PhIP when added at 10%, and increased harman and norharman at 3% and 10%. Rosemary and sage, recognized

as remarkably active antioxidant spices (actually herbs), were not the most effective, suggesting that inhibition does not totally depend on the antioxidant activity of the ingredient but on its molecular conformation.