Occurrence of Heterocyclic Amines in Muscle Foods and Mitigation with Antioxidants

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Heterocyclic Amines

- Heterocyclic amines (HCAs) are mutagenic and carcinogenic compounds that are present at ppb levels in muscle foods cooked at high temperature.

- HCAs form via Maillard reaction with creati(ni)ne, amino acids and sugars originally found in muscle foods.

- To date, more than 20 HCAs have been isolated from different cooked muscle foods.
HCAs and Cancer

International Agency for Research on Cancer (IARC - WHO)
Classifies 8 of known HCAs (MeIQ, MeIQx, PhIP, AαC, MeAαC, Trp-2, Glu-p-1) as possible human carcinogens and IQ as a probable human carcinogen.

USA National Toxicology Program (Health and Human Services)
11th Report on Carcinogens:
Classifies 4 HCAs (IQ, MeIQ, MeIQx, and PhIP) as "reasonably anticipated to be human carcinogens."
HCAs and Cancers

Epidemiological Studies - various results

The high intake of well-done meat and high exposure to meat carcinogens, particularly HCAs, may increase the risk of human cancers.

- Colorectal cancer
- Breast cancer
- Prostate cancer
- Pancreatic cancer

Heterocyclic Amines - types

- Aminoimidazole-azaarenes (AIAs) – Polar
  - Produced by moderately high heating – typical cooking/frying. Most studied group.

- Amino-carbolines (ACs) - Polar
  - Pyrolysis of amino acids and proteins at temperature above 575 °F (300 °C) higher temperatures

- Harman and Norharman - Carbolines - Nonpolar - not mutagenic
  - May still be toxic
### Common HCAs in Foods

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>R&lt;sub&gt;1&lt;/sub&gt;</th>
<th>R&lt;sub&gt;2&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>IQ</td>
<td>H</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>MeIQ</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>DiMeIQx</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>H</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;</td>
</tr>
<tr>
<td>PhIP</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(Murkovic 2004)
Formation mechanism of HCAs
via Maillard reaction

PhIP similar mechanism

Vitaglione and Fogliano (2004)
HCAs in Foods

- Meat and fish products cooked by frying, grilling and broiling (especially in the surface part)
  
  PhIP > MeIQx > DiMeIQx > IQ, MeIQ
  (nd – 50 ng/g)  (nd – 8 ng/g)  (nd – 6 ng/g)  (nd – 4 ng/g)

- Pan residues

- Grill scrapings

(Knize and Felton 2005)
Factors affecting HCA formation

- Cooking time and temperature
- Cooking methods - frying, broiling, grilling
- Concentration of precursors (creatine/creatinine, amino acid, sugar)
- Moisture content - low
- Lipid content - lower
- Meat characteristics (animal species, muscle type, aging, meat quality)
Cooking Methods

Effect of cooking methods on HCA formation in cooked meat products

(Chen and Chiu 1998)
HCAs (PHIP) in fried beef patties, cooked at different temperatures/times  (modified from: Thomson, 1999)
Inhibition of HCAs

• Modifying cooking methods
  - cook at lower temperature/time
  - microwave pre-cooking

• Addition of antioxidants
  - synthetic antioxidant (BHA, BHT, PG, TBHQ)
  - natural antioxidant polyphenolics from a wide variety of sources (fruit/plant and their extracts)

• Improving water-holding capacity
  - water-binding compounds (carbohydrate, phosphate)
Heterocyclic Amine Content in Commercial Ready to Eat Meat Products

Supported by the American Meat Institute Foundation and National Pork Board

Abstract presented at IFT Meeting 2009

Objective

To estimate the amount of HCAs in commonly consumed RTE meat products

- Hot dog beef
- Hot dog beef-pork-turkey
- Deli roast beef
- Deli ham
- Deli turkey
- Pepperoni
- Fully cooked bacon
- Rotisserie chicken
Sample Preparation

followed the package directions

Hot dog beef, Hot dog beef-pork turkey
Wrapped in a paper towel and heated in a microwave for 35 s

Fully-cooked bacon
Heated in microwave for 30 s

Pepperoni (from frozen pizza)
A frozen pizza heated in an oven at 400 °F for 23 min

Analyzed as obtained
chicken - skin and meat were separated
Conclusion

• HCA levels in RTE meat products are very low (less than 1 ng/g), except for rotisserie chicken skin.
  
  - Low-temperature manufacturing process (165-180 °F)
  - Presence of ingredients and additives that inhibit HCA formation (e.g. salt, phosphate, spices)

• Consumption of RTE meat products contributes very little to HCA intake.
Heterocyclic Amine Content in Cooked Meat Products

Supported by the American Meat Institute Foundation and National Pork Board

Abstract presented in part at IFT Meeting 2010
Currently under review for publication
Objective

To determine the HCA content in cooked meat products prepared by various cooking methods that are preferred by U.S. meat consumers. Previous studies but products have changed over the years

Choice of meat samples in this study >>

Exponent Inc. conducted Internet-based survey of U.S. consumers’ preference for method of cooking and degree of doneness of meat and fish.
# Cooking conditions

<table>
<thead>
<tr>
<th>Sample</th>
<th>Types/cuts of meat</th>
<th>Cooking method</th>
<th>Cooking temperature</th>
<th>Desire internal temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef</td>
<td>Top loin</td>
<td>Frying</td>
<td>400 °F</td>
<td>135 °F for medium-rare 160 °F for well-done</td>
</tr>
<tr>
<td></td>
<td>Top loin</td>
<td>Oven-broiling</td>
<td>450 °F</td>
<td>135 °F for medium-rare 160 °F for well-done</td>
</tr>
<tr>
<td>Pork</td>
<td>Top loin</td>
<td>Frying</td>
<td>400 °F</td>
<td>160 °F</td>
</tr>
<tr>
<td></td>
<td>Bacon</td>
<td>Frying</td>
<td>342 °F 3 min/side</td>
<td>*cook 3 slices each time</td>
</tr>
<tr>
<td>Chicken</td>
<td>Breast with and without skin</td>
<td>Frying</td>
<td>400 °F</td>
<td>160 °F</td>
</tr>
<tr>
<td>Fish</td>
<td>Catfish, Salmon, Tilapia</td>
<td>Frying</td>
<td>400 °F</td>
<td>145 °F</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oven-baking</td>
<td>350 °F</td>
<td>145 °F</td>
</tr>
</tbody>
</table>
## Results

<table>
<thead>
<tr>
<th>HCA levels</th>
<th>Cooked meat products</th>
<th>Total HCAs (ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low ( &lt; 5 ng/g)</td>
<td>Medium-rare fried beef</td>
<td>2.73</td>
</tr>
<tr>
<td></td>
<td>Fried chicken with skin</td>
<td>3.13</td>
</tr>
<tr>
<td>Intermediate (5 -10 ng/g)</td>
<td>Well-done broiled beef</td>
<td>6.04</td>
</tr>
<tr>
<td></td>
<td>Fried chicken without skin</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Baked fish</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Well-done fried beef</td>
<td></td>
</tr>
<tr>
<td>High ( &gt; 10 ng/g)</td>
<td>Fried fish</td>
<td>11.57</td>
</tr>
<tr>
<td></td>
<td>Fried pork</td>
<td>13.91</td>
</tr>
<tr>
<td></td>
<td>Fried bacon</td>
<td>17.91</td>
</tr>
</tbody>
</table>

Conclusions

• HCA content in cooked meat depends on type of meat, cooking methods, and cooking time/temperature.

• Controlling cooking time and temperature can minimize the HCA formation.
The Use of Antioxidants and Marinades to Inhibition of Heterocyclic Amines

Abstract presented in part at IFT Meeting 2007
Funded in part by Mitsubishi Chemical


Introduction

- Wide variety of research on the role of antioxidant rich plant sources inhibition of HCA formation.
- Tea, chocolate, wine, fruits, and spices have been studied. - All are rich polyphenolics which can block the reactions via several mechanisms
- Rosemary/oregano extracts are frequently use because easy to extract polyphenolics from volatiles
- Currently used in fresh and processed/frozen products, often labeled as ‘natural flavor’
How - Methods

- Add antioxidant/extract directly to product (ground beef), apply to surface, inject or marinade
- Cook on thermostat-controlled electric grill at 375-400 °F for various times
- Samples grind and analyze via HPLC
- Analyze the spice/extracts for antioxidant levels by HPLC
Objectives

- To investigate the influence of commercial marinades containing natural antioxidants on the reduction of HCAs on grilled beef steaks.

- Finding the correlation of the antioxidative components in spices and HCAs formation.
Materials & Methods

• Marinated steaks were treated with each of 3 marinades separately as labeled for one hour at 4 °C before grilling.
• Controls and base formulations
• Meat samples (eye round steak) were grilled in a thermostat-controlled Teflon coated electric grill at 400 °F (204 °C) for 5 minutes each side.
• 2-4 mm thickness of steaks removed for analysis.
# Marinades

<table>
<thead>
<tr>
<th>Marinade #1</th>
<th>Base Ingredients</th>
<th>Spices</th>
<th>Composition of liquids to formulate marinade</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>salt, sugar, caramel color</td>
<td>thyme, red pepper, black pepper, allspice, rosemary, chives</td>
<td>1/4 cup water, 2 tbs soybean oil, 2 tbs vinegar</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Marinade #2</th>
<th>Base Ingredients</th>
<th>Spices</th>
<th>Composition of liquids to formulate marinade</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>salt, sugar maltodextrin, silicon dioxide</td>
<td>paprika, red pepper, oregano, thyme, black pepper, garlic, onion,</td>
<td>2 tbs water, 1/4 cup soybean oil, 2 tbs vinegar,</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Marinade #3</th>
<th>Base Ingredients</th>
<th>Spices</th>
<th>Composition of liquids to formulate marinade</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>salt, sugar maltodextrin, modified corn starch, whey solids, soy protein, wheat gluten, calcium silicate</td>
<td>oregano, basil, garlic, onion, jalapeno pepper, parsley, red pepper</td>
<td>1/3 cup water, 3 tbs soybean 3 tbs vinegar,</td>
</tr>
</tbody>
</table>
HCAs in unmarinated and marinated steaks

- MeIQ
- MeIQx
- PhIP
- Harman
- Norharman

Percent of controls
Level of antioxidants in different marinades

- Rosmarinic Acid
- Carnosol
- Carnosic acid

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>Marinade # 1</th>
<th>Marinade # 2</th>
<th>Marinade # 3</th>
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<tbody>
<tr>
<td>0</td>
<td>10</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
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<tr>
<td>45</td>
<td>45</td>
<td>25</td>
<td>45</td>
</tr>
</tbody>
</table>
Enhancement and Commercial Products

Supported by the American Meat Institute Foundation and National Pork Board
Summary

Enhancement of fresh meat - addition of salt and phosphate (7-15%), significant reduction in HCA levels

- Pork 50% reduction
- Commercially enhanced and marinated products:
  - With 12-30% enhancement see 30-60% reductions
  - Some is clearly tied to water retention but also to presence of antioxidants (spices)
Reciprocation

- Where do we go from here?
  - Revised products with more enhancement?
  - Add more antioxidants?
  - Promote antioxidants in meat products.
  - How to deal with “bad” press? Do we ignore or should there be more discussion and education?