High Concentrations of the Carcinogen 2-Amino-1-methyl-6-phenylimidazo-[4,5-b]pyridine (PhIP) Occur in Chicken but Are Dependent on the Cooking Method

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Abstract

Heterocyclic aromatic amines (HAAs) are mutagenic and carcinogenic compounds found in meats cooked at high temperatures. Although chicken is consumed in large quantities in the United States, there is little information on its HAA content. The objective of this study was to measure the five predominant HAAs (IQ, MeIQ, MeIQx, DiMeIQx, and PhIP) in chicken cooked by various methods to different degrees of doneness. Chicken breasts were panfried, oven-broiled, or grilled/barbecued. Whole chickens were roasted or stewed. Skinless, boneless chicken breasts were cooked to three degrees of doneness: just until done, well done, or very well done. High levels of PhIP (ranging from 12 to 480 ng/g cooked meat) were found in chicken breasts when panfried, oven-broiled, and grilled/barbecued, but not in whole roasted or stewed chicken. PhIP concentration increased in skinless, boneless chicken breast with longer cooking time, higher internal temperature, and greater degree of surface browning. PhIP concentration was also high in chicken breasts cooked with skin and bones. MelQx and DiMeIQx levels increased with the degree of doneness, whereas IQ and MeIQ were not detectable in any of these chicken samples. Certain cooking methods produce PhIP, a known colon and breast carcinogen in rodents and possibly a human carcinogen, at substantially higher levels in chicken than has been reported previously in red meat.

Introduction

HAAs, a family of compounds known to be highly mutagenic in vitro, are formed in meats cooked at high temperatures (1-3). PhIP, one of the most abundant HAAs in cooked meat, produces colon and mammary gland tumors in rodents (4-6), especially in conjunction with diets high in fat (7, 8). The IARC concludes that "there is sufficient evidence in experimental animals for the carcinogenicity of PhIP" and that "PhIP is possibly carcinogenic to humans" (9). Furthermore, the results of several epidemiological studies suggest that consumption of well done or well-browned red meat, potential surrogates for HAAs, is associated with colorectal and breast cancers (10-13). To date, it has been widely assumed that red meat is the primary source of exposure to HAAs. In a preliminary study, however, Watabayashi et al. (14) reported that broiled chicken contained twice the level of PhIP (38.1 ng/g cooked meat) when compared to broiled beef (15.7 ng/g cooked meat). Since over 20% of the meat eaten in the United States is poultry (15), we measured the levels of HAAs in chicken cooked by a variety of methods to different degrees of doneness.

Materials and Methods

Three types of chicken were purchased from a local supermarket: skinless, boneless chicken breasts; breasts with skin and bones; and whole chicken. Whole chickens were roasted or stewed. Chicken breasts were either panfried, oven-broiled, or grilled/barbecued. Whole chickens were roasted or stewed.

The chicken was cooked by nutritionists at the Human Nutrition Research Center, United States Department of Agriculture, Beltsville, MD. For panfried and oven-broiled chicken, one skinless, boneless breast was cooked in each of six sessions (Table 1). The number and cooking levels of skinless boneless chicken breast samples that were grilled/barbecued followed: three breasts were cooked in one session, just until done; three breasts were cooked in each of two sessions, well done; six breasts were cooked in one session, very well done. For chicken breasts with skin and bones, two pieces were cooked in each of three sessions. One whole chicken was either roasted or stewed in each of five sessions. The chicken breasts were either panfried, oven-broiled, or grilled/barbecued. Whole chickens were roasted or stewed. The chicken was cooked by nutritionists at the Human Nutrition Research Center, United States Department of Agriculture, Beltsville, MD. For panfried and oven-broiled chicken, one skinless, boneless breast was cooked in each of six sessions (Table 1). The number and cooking levels of skinless boneless chicken breast samples that were grilled/barbecued followed: three breasts were cooked in one session, just until done; three breasts were cooked in each of two sessions, well done; six breasts were cooked in one session, very well done. For chicken breasts with skin and bones, two pieces were cooked in each of three sessions. One whole chicken was either roasted or stewed in each of five sessions. The chicken breasts were either panfried, oven-broiled, or grilled/barbecued. Whole chickens were roasted or stewed.
cooked until the internal temperature reached 78 to 81°C, there was little or no browning on the surface, with no traces of pink meat or juice. For well done and very well done categories, the internal temperature was at least 5°C higher than the well done category.

We used the most common cooking methods used in the United States: panfry, oven-broil, grill/barbecue, roast, or stew (we have reported deep-fried chicken elsewhere (17)). Panfried chicken breasts (with or without skin and bones) were cooked in a Teflon-coated frying pan with 2 tablespoons of oil. The temperature on the pan surface was monitored with a surface thermometer (PTC; Pacific Transducer Corp., Los Angles, CA). Oven-broiled chicken breasts (with or without skin and bones) were cooked in a commercial gas range broiler with the meat placed 5 inches away from the heat source. The surface temperature was monitored with a thermocouple probe near the surface of the broiling pan. Grilled/barbecued chicken breasts (with or without skin and bones) were prepared on a home propane gas barbecue unit with ceramic briquettes (Sunbeam Model 44M39, 27 1/2" x 15", 44,000 BTU). The surface temperature was recorded with a surface thermometer on the grill surface. Whole chicken was roasted in a gas oven heated to 175°C and cooked until the internal temperature reached 85°C in the thickest part of the breast. gravy was made from the collected drippings, and the five sets of gravy were combined and assayed for HAAs. Stewed whole chicken was covered with water in a large pot and simmered until the leg joint became soft/moveable and the skin started to pull away from the leg bone.

Detailed information on the cooking methods and the criteria used to define different degrees of doneness is shown in Table 1. Other information gathered to further define the cooking methods included weight of chicken before and after cooking to calculate the percentage loss of weight with cooking and the total cooking time. The cooking time for just until done chicken was similar to cooking times recommended for various recipes (18).

The levels of IQ, MeIQ, MeIQx, DiMeIQx, and PhIP were measured in each of the composite samples extracted by solid phase extraction and HPLC according to the procedure of Gross and Gruter (19), which has been described in detail by Kuzie et al. (17). The samples were measured by investigators without the knowledge of cooking method or degree of doneness. Replicate samples containing relatively low and high HAAs were used as QC and were interspersed throughout the analysis to check for reproducibility. The HAA QC samples were made from hamburger cooked at low temperature (containing low levels of HAAs—low QC) or hamburger patties cooked at high temperature (containing high levels of HAAs—high QC). The average concentrations of MelIQx, PhIP, and DiMeIQx found in the high QC (n = 13) were: 14.0 (0.20), 1.7 (0.40) ng/g, respectively (17). IQ and MelIQ were not detectable in the QC samples.

**Results**

Chicken breasts without skin and bones when panfried, oven-broiled, or grilled/barbecued contained MelIQx, DiMeIQx, and PhIP but no detectable levels of IQ or MelIQ. Both the cooking method and degree of doneness determined the type and amount of HAAs formed, as shown in Fig. 1. PhIP levels were the highest of the three HAAs formed in all samples. Panfried chicken breasts without skin and bones contained substantial levels of PhIP, even when cooked to just until done (12 ng/g), which increased to 70 ng/g for very well done meat. Broiled chicken breasts without skin and bones contained higher levels than panfried chicken with 150 ng/g in the very well done category. Grilled/barbecued chicken breasts without skin and bones contained the highest levels of PhIP, ranging from 27 ng/g in the just until done sample, to 140 ng/g in the well done sample, and 480 ng/g in the very well done sample.

Panfried chicken breasts without skin and bones contained low levels of MelIQx at every degree of doneness. Broiled chicken breasts without skin and bones in the just until done and well done categories did not contain detectable MelIQx, but the very well done samples contained a small amount. The just until done grilled/barbecued chicken breasts without skin and bones contained nondetectable levels of MelIQx, well done breasts had 2 ng/g, while the very well done sample contained 9 ng/g (Fig. 1).

In these same panfried samples, DiMeIQx increased with degree of doneness with the lowest amount found in the just until done category and highest level in the very well done category. Oven-broiled breast meat did not contain detectable levels of DiMeIQx. Grilled/barbecued chicken breasts had no DiMeIQx in just until done samples, while

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**Table 1** Characteristics of chicken cooked by different methods to varying degrees of doneness

<table>
<thead>
<tr>
<th>Chicken samples, cooking method, and degree of doneness</th>
<th>No. of samples (sessionx ∗ no. of pieces)</th>
<th>Weight loss during cooking (%)</th>
<th>Surface temperature (°C)</th>
<th>Internal temperature (°C)</th>
<th>Total cooking time (min)</th>
<th>Surface browning/charring</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken breast without skin and bone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Panfry</td>
<td>6 (6 × 1)</td>
<td>25</td>
<td>76</td>
<td>14</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Well done</td>
<td>6 (6 × 1)</td>
<td>35</td>
<td>82</td>
<td>28</td>
<td>Moderate</td>
<td></td>
</tr>
<tr>
<td>Very well done</td>
<td>6 (6 × 1)</td>
<td>43</td>
<td>90</td>
<td>36</td>
<td>Dark brown</td>
<td></td>
</tr>
<tr>
<td>Oven-Broil</td>
<td>6 (6 × 1)</td>
<td>31</td>
<td>80</td>
<td>9</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Just until done</td>
<td>6 (6 × 1)</td>
<td>42</td>
<td>91</td>
<td>14</td>
<td>Moderate</td>
<td></td>
</tr>
<tr>
<td>Well done</td>
<td>6 (6 × 1)</td>
<td>51</td>
<td>98</td>
<td>17</td>
<td>Dark brown</td>
<td></td>
</tr>
<tr>
<td>Very well done</td>
<td>6 (6 × 1)</td>
<td>59</td>
<td>83</td>
<td>40*</td>
<td>Moderate</td>
<td></td>
</tr>
<tr>
<td>Grill/Barbecue</td>
<td>3 (1 × 3)</td>
<td>28</td>
<td>78*</td>
<td>10</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Just until done</td>
<td>6 (2 × 3)</td>
<td>45</td>
<td>83</td>
<td>40*</td>
<td>Moderate</td>
<td></td>
</tr>
<tr>
<td>Well done</td>
<td>6 (1 × 6)</td>
<td>59</td>
<td>99</td>
<td>43</td>
<td>Charred</td>
<td></td>
</tr>
<tr>
<td>Very well done</td>
<td>6 (1 × 6)</td>
<td>260</td>
<td>43</td>
<td>62</td>
<td>Moderate</td>
<td></td>
</tr>
<tr>
<td>Chicken breast with skin and bone* well done</td>
<td>6 (3 × 2)</td>
<td>30</td>
<td>78</td>
<td>62</td>
<td>Moderate</td>
<td></td>
</tr>
<tr>
<td>Panfry</td>
<td>6 (3 × 2)</td>
<td>53</td>
<td>96</td>
<td>43</td>
<td>Moderate</td>
<td></td>
</tr>
<tr>
<td>Oven-Broil</td>
<td>6 (3 × 2)</td>
<td>42</td>
<td>83*</td>
<td>63</td>
<td>Moderate</td>
<td></td>
</tr>
<tr>
<td>Grill/Barbecue</td>
<td>6 (3 × 2)</td>
<td>191 (500 during flashing)*</td>
<td>83*</td>
<td>63</td>
<td>Moderate</td>
<td></td>
</tr>
<tr>
<td>Whole chicken</td>
<td>5 (5 × 1)</td>
<td>175</td>
<td>85</td>
<td>Moderate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Roast</td>
<td>5 (5 × 1)</td>
<td>100</td>
<td></td>
<td></td>
<td>Moderate</td>
<td></td>
</tr>
</tbody>
</table>

* A session was when the whole cooking procedure was started anew with clean pans, etc.
* Chicken with skin and bones were larger pieces; therefore, they were cooked for longer times to achieve the same level of doneness as skinless, boneless chicken.
* A session was when the whole cooking procedure was started anew with clean pans, etc.
* There was flashing of flames due to dripping fat during the cooking of chicken breasts with skin and bones; therefore the lid was left p during the preparation of these samples.
* There was flashing of flames due to dripping fat during the cooking of chicken breasts with skin and bones; therefore the lid was left p during the preparation of these samples.

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well done and very well done chicken breasts contained small amounts (Fig. 1).

Chicken breast with skin and bones cooked well done also contained appreciable levels of PhIP but had undetectable levels of both MelQx and DiMelQx. The PhIP content of these chicken samples by cooking method was 25 ng/g for panfried, 131 ng/g for oven-broiled, and 36 ng/g for grilled/barbecued.

Roasted chicken, stewed chicken, and chicken gravy contained no detectable levels of HAAs.

Discussion

High levels of PhIP were found in chicken that was panfried, oven-broiled, or grilled/barbecued but not in roasted or stewed chicken. In the former samples, PhIP levels were much higher than amounts reported previously in red meats (3). In our earlier study, very well done panfried hamburgers cooked to maximize the production of these compounds contained 32.8 ng/g of PhIP (20). The comparable preparation of panfried, very well done chicken breast contained more than double the amount of PhIP at 70 ng/g meat. In this study, when steak and chicken samples were grilled/barbecued to very well done, the steak contained 30 ng/g, while chicken contained 480 ng/g of PhIP.

PhIP concentration increased in chicken breasts with greater degree of doneness. People may routinely eat chicken well done, samples which contain substantial quantities of PhIP as compared to other types of cooked meat samples (20–22). The amino acid, sugar, creatinine, or moisture content of the chicken breast may have contributed to the high levels of PhIP found in this study. The cooking conditions that lead to enhanced formation of PhIP need to be further investigated since PhIP has been implicated in the etiology of colon and mammary gland tumors in the rodent model (4, 7) and may contribute to risk of these cancers in humans.

These findings are important for epidemiological studies of HAAs and cancer. White meat (chicken and fish) consumption, in contrast to red meat consumption, has not been consistently associated with excess colon cancer risk (23) and, in some cases, has even been found to be protective (24, 25). Thus, if cooked chicken contains high levels of PhIP, then PhIP may not be a human carcinogen, or its association with cancer may have been attenuated because chicken preparation techniques have not been considered in detail by previous studies. Based upon our data, people who consume chicken cooked by roasting/baking, stewing/braising, or deep frying (17) receive little PhIP exposure, while those who consume chicken cooked by panfrying, oven-broiling, and grilling/barbecuing may have substantial exposure. Furthermore, chicken and fish consumption may be confounded with other healthy aspects of diet such as higher consumption of fruit/vegetables, fiber, and lower intake of fat.

Red meat, as noted above, has been associated with an increased risk of colon cancer in many studies (10, 11, 24, 25). People who eat predominantly red meat may cook it by methods which produce high levels of HAAs such as panfrying, oven-broiling, or grilling/barbecuing. In addition, red meat consumption may be associated with other factors which increase cancer risk such as fat. For example, animal studies show that the effect of HAAs on the development of colon and mammary gland tumors may be modified in the presence of a high fat diet (7, 8). Since a diet high in fat is more strongly associated with red

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*Unpublished data.*
meat intake than with the intake of chicken or fish, the effect of HAA s may be manifested only among individuals consuming predominantly red meat.

To investigate the association of HAs and cancer, all sources of these compounds should be used to create an exposure index that can accurately categorize subjects in epidemiological studies. To date, only surrogates of red meat HAA exposure (doneness or brownness) have been used in these studies. All types of meat, i.e., beef, pork, chicken, and fish, need to be incorporated in a matrix with cooking method and level of doneness. To this end, we are currently developing a database for meats cooked by different methods to varying levels of doneness to link with a meat cooking module within a food frequency questionnaire.lov

Although the study presented here is the most comprehensive to date on chicken, further studies are needed to better evaluate human dietary exposure to HAAs. Multiple chicken samples were cooked and made into a single composite sample, which was then analyzed. Thus, data on variability in HAA content within samples cooked by the same technique to the same degree of doneness are not available. In this study, we examined the production of HAAs in white chicken meat only. Further studies are needed to investigate the effect of cooking and doneness on HAA production in the dark meat portions of chicken (legs, thighs, and wings). Moreover, cooking conditions could not always be tightly controlled so that the internal temperature, external appearance, and total cooking time did not increase consistently from one degree of doneness to the next. Conditions for panfrying were the easiest to control, while those for grillling/barbecuing were the most difficult. Finally, chicken samples were cooked to different degrees of doneness and browning in a nutrition research facility. To judge the representativeness of these samples, there is a need to document usual home-cooking conditions.

In summary, we found that certain cooking methods produce relatively high levels of PhIP in chicken. Although the link between consumption of HAAs and excess cancer risk in humans has yet to be demonstrated, these compounds are established animal carcinogens (9). As such, it may be prudent to minimize exposure to PhIP when eating chicken. This can be achieved by roasting or stewing chicken and by avoiding overcooking or overbrowning when panfrying, broiling, and grillling/barbecuing.

References


5 R. Sinha et al., manuscript in preparation.

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