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Abbreviations: 4,8-DiMeIQx, 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline; 7,8-DiMeIQx, 2-amino-3,7,8-trimethylimidazo[4,5-*f*]quinoxaline; HAA, heterocyclic aromatic amines; HPLC, high performance liquid chromatography; IFP, 2-amino-(1,6-dimethylfuro[3,2-*e*]imidazo [4,5-*b*])pyridine; MeIQ, 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline; MeIQx, 2-amino 3,8-

dimethylimidazo[4,5-*f*]quinoxaline; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; PRS, propylsulfonic acid silica.

Abstract--Chicken and fish samples prepared by 42 Singapore Chinese in their homes were obtained. Researchers were present to collect data on raw meat weight, cooking time, maximum cooking surface temperature, and cooked meat weight. Each participant prepared one pan-fried fish sample and two pan-fried chicken samples, one marinated, one not marinated. The cooked samples were analyzed for five heterocyclic aromatic amine (HAA) mutagens, including MeIQx (2-amino 3,8-dimethylimidazo[4,5-*f*]quinoxaline); 4,8-DiMeIQx (2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline); 7,8-DiMeIQx (2-amino-3,7,8-trimethylimidazo[4,5-*f*]quinoxaline); PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine), and IFP (2-amino-(1,6-dimethylfuro[3,2-*e*]imidazo [4,5-*b*])pyridine). A paired Student's t-test showed that marinated chicken had lower concentrations of PhIP ($p<0.05$), but higher concentrations of MeIQx ($p<0.05$) and 4,8-DiMeIQx ($p<0.001$) than non-marinated chicken, and also that weight loss due to cooking was less in marinated chicken than in non-marinated chicken ($p<0.001$). Interestingly, the maximum cooking surface temperature was higher for fish than for either marinated or non-marinated chicken ($P<0.001$), yet fish was lower in 4,8-DiMeIQx per gram than marinated or non-marinated chicken ($p<0.001$), lower in PhIP than non-marinated chicken ($P<0.05$), and lost less weight due to cooking than either marinated or non-marinated chicken ($P<0.001$). Fish was also lower in MeIQx and 7,8-DiMeIQx than marinated chicken ($P<0.05$). This study provides new information on HAA content in the Singapore Chinese diet.

Introduction

Heterocyclic aromatic amines (HAA) are compounds that form naturally in muscle meats when exposed to heat during the cooking process (Sugimura 1997). Known for their potent mutagenic response on the Ames/*Salmonella* test (Ames et al. 1975) and in Chinese hamster ovary cells (Wu et al. 1997), HAA also have been linked to cancer in laboratory rodents and primates (Gold et al. 1994; Snyderwine et al. 1997; Nagao et al. 2002; Nakagama et al. 2002). The U.S. Department of Health and Human Services recently listed three HAA, MeIQ, MeIQx, and PhIP, as "reasonably anticipated to be human carcinogens" based on a body of evidence from *in vivo* studies and human epidemiological data (NIEHS 2005). The HAA have been studied extensively for more than twenty years, using methods that were developed for their isolation from the food matrix and various analytical protocols designed to detect, identify, and quantify them (Murray et al. 1988; Gross 1990; Richling et al. 1996). Several of them, especially MeIQx and PhIP, are frequently detected in commonly consumed meats, with concentrations typically ranging from 0.1 to hundreds of nanograms per gram of cooked meat (Knize et al. 1999).

The relationship between dietary HAA exposure and cancer risk is still not fully understood, but interest in this matter has been growing and has been reviewed recently (Knize and Felton 2005). Higher exposures to HAA are more likely from consuming well-done meats, and epidemiological studies to examine the relationship between cancer incidence and a preference for well-done meats have shown, in several cases, positive correlations with colorectal (Sinha et al. 2001), breast (Sinha et al. 2000), pancreatic (Anderson et al. 2002), and prostate cancer (Bogen and Keating 2001). Not all such studies

have shown a positive correlation, however (Muscat and Wynder 1994; Ambrosone et al. 1998; Augustsson et al. 1999). Several recent studies have focused on both home-cooked and restaurant-cooked meats, as being most representative of actual exposure to HAA within populations. A large survey of meats cooked at home by members of a cohort within the United States yielded valuable information about HAA concentrations (Keating et al. 2000; Knize et al. 2002), as did a study of commonly consumed home-cooked meats obtained from households in Spain (Busquets et al. 2004). Ristic, et al. reported on their HAA analysis of several home-cooked and commercially-prepared meats typical of those consumed by Austrians (Ristic et al. 2004). Restaurant foods have been analyzed for HAA content in several studies including the United States (Knize et al. 1995; Knize et al. 1998; Pais et al. 2000), Germany (Richling et al. 1998) and Canada (Klassen 2002). Additionally, Solyakov and Skog reported on HAA in commercially barbecued chicken purchased in supermarkets in Sweden (Solyakov and Skog 2002).

While the information derived from such studies serves as an essential base of knowledge from a public health standpoint, it also can provide clues to understanding the factors that affect HAA formation and can indicate means of reducing or eliminating these compounds. Numerous laboratory studies have investigated the effects of cooking temperature and time, with a clear correlation generally being seen between these determinants and HAA concentration (Knize et al. 1994; Skog et al. 1995). Several studies have examined other factors that might affect HAA formation, such as pretreatment with marinades (Tikkanen et al. 1996; Salmon et al. 1997; Nerurkar et al. 1999), addition of vitamins, herbs or spices (Murkovic et al. 1998; Balogh et al. 2000), combination of fruit or vegetables with ground meat (Britt et al. 1998; Kato et al. 1998), and use of techniques such as microwave cooking

(Felton et al. 1994; Chiu et al. 1998), convection roasting (Skog et al. 2003), or frequent turning of meat (Salmon et al. 2000). Inherent characteristics of meats, such as animal source (Vikse and Joner 1993; Pais et al. 1999; Olsson et al. 2002), and fat and water content (Johansson and Jägerstad 1994) have also been considered, relative to the HAA levels that form in them when cooked.

Some of these cooking practices are used more commonly in the cuisines of certain cultures and may, conceivably, impact those populations by presenting different degrees of exposure to HAA. An abundance of research has been done on the relationship between cancer risk and HAA present in typical Western cuisine, with its greater emphasis on meats in general and red meats in particular, but less is known about Asian cuisine with regard to HAA content. There is growing interest by researchers to examine Asian diets and cancer risk. Japanese researchers reported that the major dietary source of HAA on mainland Japan is grilled fish (Kobayashi et al. 2002). While, to date, little is known about HAA content in the typical diet of the Singapore Chinese population, the recent publication of a 24-hour dietary recall study of Chinese residents of Singapore provided a springboard for conducting a comprehensive survey of foods. In their study, the authors found that nearly 90% of the meat intake of that population comprised fish, pork, and poultry, and they documented cooking methods and portion sizes in their interviews (Koh et al. 2005). Subsequent to the work of Koh et al., Wong et al. conducted a 24 hour dietary recall survey of 497 Singapore Chinese coupled with an HAA analysis of 25 pooled samples of commonly consumed meats, the first such study to address the issue of dietary exposure to HAA in the Singapore Chinese population (Wong et al. submitted).

Following the direction in which the work of Koh et al. and Wong et al. pointed, we obtained pan-fried fish and chicken samples prepared by 42 Chinese Singapore residents in their own homes and analyzed them for HAA content. Our objectives were to correlate HAA concentrations in a representative sampling of Chinese home-cooked meats with known determinants (cooking duration, maximum surface temperature, weight loss due to cooking, and meat type); to compare HAA levels in marinated chicken cooked under domestic conditions with those of chicken not marinated before cooking; and to measure HAA concentrations in the diet of a sampling of Chinese Singaporeans, in order to compare their exposures to the HAA exposures estimated for other populations around the world. The information derived from this study contributes to a repository of HAA information relevant to Chinese cooking and dietary practices and important general knowledge for understanding the connection between cooking practices, carcinogens, and cancer risk.

Materials and Methods

Meat Samples and Cooking Procedures

Fish and chicken samples were provided to 42 Singapore residents of Chinese ethnicity who agreed to participate in this study. The chicken samples were whole boneless thigh with skin, and the fish samples were whole red snapper with skin. Each household's cook was instructed to marinate one chicken thigh with their "usual" marinade before cooking and to cook the other chicken thigh without marinating it first. Table 1 lists the major ingredients of the various marinades and the percentage of households that included each ingredient. The participants were asked to cook the samples whole (not chopped) without removing the skin. All of the fish and chicken samples were pan-fried in a shallow layer of preheated cooking oil, with each household cook using his or her usual cooking technique. All participants cooked on gas appliances and were asked to use their usual cookware, of which 27 (64.3%) used cast-iron woks, 6 (14.3%) used heavy stainless steel frying pans, 4 (9.5%) used steel or aluminum woks, and 5 (11.9%) used non-stick frying pans. Before, during, and after the cooking process for each sample, scientists monitored several cooking parameters, recording the raw weight of each sample, the temperature of the cooking oil just prior to placing the sample in the wok or frying pan, the change in temperature of the oil just after placing the sample in it, the maximum temperature that the oil reached during the cooking of each sample, the duration in minutes that the sample cooked, and, finally, each sample's cooked weight. The edible portion of each cooked meat was coarsely ground in a blender, and representative samples of approximately 20 grams each were frozen at –30°C and later stored at –20°C prior to analysis for HAA content.

HAA extraction and HPLC analysis

All reagents and solvents used in these procedures were analytical or HPLC grade. The extractions and high performance liquid chromatography (HPLC) analyses of these 123 meat samples were carried out over a period of 3 months. The extraction procedure according to Gross and Grüter (Gross and Grüter 1992) was performed. Briefly, 5 grams of each sample was homogenized in 15 grams 1N NaOH. Half of one of the sample homogenates of each day's set was supplemented ("spiked") with a mixture of 5 HAA standards identical to the compounds of interest in this analysis. IFP was a natural product isolated from a creatine-added meat mixture (Pais et al. 2000); all others were from Toronto Research Chemicals Inc. (Downsview, ON, Canada). Standards' concentrations were determined by UV absorbance measurement and calculated using established extinction coefficients (Salmon et al. 2000). IFP, for which no extinction coefficient has yet been established, was quantified as described (Pais et al. 1999). The other half of the homogenate remained unspiked. The weighed homogenates were mixed with diatomaceous earth-based bulk material for liquid-liquid extractions (Chem Tube-Hydromatrix, Varian, Harbor City, CA), and HAA were extracted using a mixture of methylene chloride: toluene (95:5) onto propylsulfonic acid silica (PRS, 500mg) solid phase extraction cartridges (Varian). The PRS cartridges were washed, the analytes transferred to 100mg C_{18} solid phase extraction cartridges (Varian), and then eluted with 0.8 ml of a 9:1 solution of methanol and ammonium hydroxide. The dissolved extracts were dried at 40°C under a stream of N_2, dissolved in 50μl of a 50:50 mixture of methanol and HPLC mobile phase, and 40μl aliquots of each dissolved extract were analyzed as described (Knize et al. 1995) by reverse phase HPLC on a Waters system with photo-diode array UV detector (Waters Corp. Milford, MA) and a Shimadzu RF-10A_{XL} fluorescence

detector (Shimadzu Corp., Kyoto, Japan) set at excitation wavelength 307nm and emission wavelength 370nm. The peaks of interest on the resulting chromatograms were identified by comparison of their retention times and their UV spectra with those of the spiked sample. Fluorescence peaks at corresponding retention times offered further confirmation of the presence of PhIP and IFP in the sample and a greater signal to noise ratio for their quantitation.

Individual HAA concentrations, expressed as nanograms HAA per gram of cooked meat, were calculated from peak areas and were corrected for incomplete recoveries using the known concentrations of the HAA spiking mixture. Percent recoveries for each compound in the spiked samples were: MeIQx (58 ± 9.9); PhIP (18 ± 5.9); 4,8-DiMeIQx (52± 10.4); 7,8-DiMeIQx (53 ± 10.8), and IFP (10 ± 4.8). Instrument detection limits for each compound within the sample matrix were: MeIQx 0.004 ng/g; PhIP 0.06 ng/g; 4,8-DiMeIQx 0.01 ng/g; 7,8-DiMeIQx 0.002 ng/g, and IFP 0.02 ng/g.

Statistical methods

All samples analyzed were grouped into 3 "treatment" groups: non-marinated chicken (n=42), marinated chicken (n=39), and fish (n=42). Complete sample sets (a fish sample and both types of chicken samples, as well as time, temperature, and sample weight loss data) were available for most of the 42 households; however, a few households provided only one or two of the meat samples, and cooking data were unavailable for one household. Therefore, in order to perform the most rigorous analysis of the data, a paired Student's t-test (two-tailed) using the SPSS statistical package (SPSS, Inc., Chicago, IL) was selected to determine statistical significance of differences in the following: the 5 individual HAA concentrations, weight loss due to cooking, maximum cooking surface temperature, and

cooking time for each treatment group. Non-marinated chicken samples were each paired with the corresponding marinated chicken samples from each household, and fish from each household were likewise paired with each type of chicken from the same household. In the few cases where there were missing samples or cooking data, the available data from that set had to be omitted from the paired Student's t-test.

Table 1: Marinade Ingredients

Ingredient	Used by number (%) of households
Ground white pepper	31 (73.8%)
Soy sauce	28 (66.7%)
Salt	17 (40.5%)
Sesame oil	15 (35.7%)
Oyster sauce	13 (31.0%)
Garlic (fresh)	11 (26.2%)
Other (unspecified)	11 (26.2%)
Ginger (fresh)	9 (21.4%)
Cornstarch	8 (19.0%)
Huatiao (cooking wine)	7 (16.7%)
Sugar	5 (11.9%)
Onion	2 (4.8%)

Results

Means and standard deviations of cooking conditions are shown in Table 2. Marinated chicken and non-marinated chicken were not significantly different in terms of maximum surface cooking temperature, but marinated chicken lost significantly less weight due to cooking than did non-marinated chicken ($p<0.001$). The maximum temperature at the cooking surface for fish was significantly higher than for both non-marinated and marinated chicken ($p<0.001$), yet it lost significantly less weight than either chicken type ($p<0.001$). There were no significant differences in cooking times for the three sample types.

Mean values and standard deviations for individual HAA concentrations in marinated chicken, non-marinated chicken, and fish are shown in Table 3. A Student's t-test showed that marinated chicken had significantly less PhIP ($p<0.05$) and significantly more MeIQx ($p<0.05$) and 4,8-DiMeIQx ($p<0.001$) than non-marinated chicken. Fish was significantly lower in PhIP ($p<0.05$) and 4,8-DiMeIQx ($p<0.001$) than non-marinated chicken and had significantly less MeIQx ($p<0.05$), 7,8-DiMeIQx ($p<0.05$) and 4,8-DiMeIQx ($p<0.001$), than marinated chicken. PhIP concentrations were no different in fish and marinated chicken ($p=0.84$).

Table 2: Cooking parameters for pan-fried chicken and fish from Singapore Chinese households

Meat type*	Weight loss (%)	Cook time (min)	Maximum surface temperature (°C)
Non-marinated Chicken	37.0 ± 7.6	7.65 ±3.33	192.5 ± 33.7
Marinated Chicken	30.8 ± 8.4[A]	7.19 ± 2.50	190.1 ± 37.2
Fish	20.9 ± 4.4[A,B]	8.24 ± 2.99	222.5 ± 32.6[A,B]

Data represent means ± standard deviations.
A: Significantly different from non-marinated chicken ($p<0.001$), Student's t-test, paired, 2-tailed.
B: Significantly different from marinated chicken ($p<0.001$), Student's t-test, paired, 2-tailed.

*Sample pairs:
For non-marinated chicken paired with marinated chicken by household, n= 38.
For non-marinated chicken paired with fish by household, n= 37.
For marinated chicken paired with fish by household, n= 36.

Table 3: Heterocyclic Amine Concentration in pan-fried chicken and fish from Singapore Chinese households

Meat type*	MeIQx	PhIP	4,8-DiMeIQx	7,8-DiMeIQx	IFP	TotalHAA
Non-marinated Chicken	0.18 ± 0.2	2.37 ± 2.9	0.11 ±0.1	0.002 ± 0.01	0.087 ± 0.2	2.75 ±3.2
Marinated Chicken	0.75 ± 1.5[A]	1.34 ± 1.9[A]	0.24 ± 0.3[A]	0.01 ± 0.03	0.11 ± 0.3	2.46 ± 3.2
Fish	0.10 ±0.28[B]	1.34 ±1.9[A]	0.02 ± 0.1[A,B]	0.0004 ± 0.002[B]	0.06 ± 0.3	1.56 ±2.3[A]

Heterocyclic amine concentrations expressed as nanograms per gram of cooked meat (means ± standard deviations).

A: Significantly different from non-marinated chicken ($p<0.05$, except $p<0.001$ for 4,8-DiMeIQx); Student's t-test, paired, 2-tailed.
B: Significantly different from marinated chicken ($p<0.05$, except $p<0.001$ for 4,8-DiMeIQx); Student's t-test paired, 2-tailed.

*Sample pairs:
For non-marinated chicken paired with marinated chicken by household, n= 39.
For non-marinated chicken paired with fish by household, n= 38.
For marinated chicken paired with fish by household, n= 37.

Discussion

This work was part of a larger ongoing effort in Singapore to determine the range of HAA concentrations to which Singapore Chinese people are exposed in their daily diet, in order to compare their HAA exposure to that estimated for people in other parts of the world. From this sampling of 42 households, HAA concentrations were found to range from barely detectible levels (less than 0.001 ng/g) to nearly 15 ng/g in some meat samples. These numbers compare favorably with HAA values measured in many similar surveys of home- or commercially-prepared foods from other parts of the world. Using PhIP in fried chicken as an example, since it is generally the most mass abundant HAA measured in cooked meats, reported values range from 2.3 to 46.9 ng/g in Spain (Busquets et al. 2004); 0.2 to 2.1 ng/g in Canada (Klassen 2002); 1.3 to 11.1 ng/g in Austria; (Ristic et al. 2004) and non-detectable levels to 1.44 ng/g in the United States (Knize et al. 1998). (It should be noted that the cooking conditions, sampling techniques, and methods of analysis varied somewhat among these surveys, however.) Similar variation in values for other meat types are reported in these and other studies, as well, but most typical values for PhIP range from less than 1 ng/g to tens of ng/g in home- or commercially-prepared meats.

The specific objectives of this study were to correlate HAA concentrations in Chinese home-cooked meats with known determinants (cooking time, temperature, and weight loss due to cooking), to investigate the effect of marinating on HAA levels in chicken, and to compare the HAA levels in two common sources of animal protein in the Chinese Singapore diet, chicken and fish, when prepared under domestic conditions. A number of conditions in this study were carefully controlled: the same types of chicken and fish samples were

provided to the participants; they were instructed to cook them whole, with skin on, to fry in a small amount of oil, to flip the meat once during cooking, and to marinate half of the chicken ahead of time; they all cooked on gas appliances, and temperature, time, and cooking weight were all monitored by trained researchers. However, this was not a controlled study as would be performed in a laboratory, in that the chicken and fish samples differed in mass and other characteristics, such as shape and coverage by skin; also, many cooking conditions varied according to the individual domestic cooks' techniques and the cooking equipment available in their homes. In addition, the participants were instructed to use their own marinade recipes. Importantly, the determination of "doneness" was left to the discretion of each cook, resulting in food that was cooked to the level that would normally be consumed in the home. This judgment of doneness could have been affected by the different appearance of marinated chicken due to browning of the marinade itself, rather than the actual degree of doneness of the meat (internal meat temperatures were not measured, nor was there any set length of cooking time). These differences probably introduced a greater degree of variation in the results than would have been seen in a strictly controlled laboratory experiment, but also yielded a more realistic range of the HAA concentrations to which people are exposed from consuming a typical meal. Some variation, particularly evident for the three imidazoquinoxaline compounds measured in the marinated chicken samples, was likely a consequence of the differences among marinades used by the individual household cooks.

Marinating chicken has been shown to affect HAA concentration in a number of controlled studies. Salmon, et al. showed a 92-99% reduction in PhIP and a statistically insignificant increase in MeIQx with increased cooking time in marinated grilled chicken (Salmon et al. 1997). Nerurkar, et al. showed reduced PhIP and MeIQx in barbecued beef

marinated with two different marinades, but increased HAA with a third marinade (Nerurkar et al. 1999). Tikkanen et al. tested four different marinades on grilled chicken and observed some increases and some decreases in mutagenic activity and in HAA levels measured by gas chromatography/mass spectrometry, depending on the marinade used and/or the cooking conditions (Tikkanen et al. 1996). The above-mentioned marinade experiments all utilized grilling over hot coals as the cooking method, whereas the meat samples in this survey were all pan-fried. In this present study of domestically-prepared meats, the difference in cooking method (pan-frying) introduces a new factor to consider, and the great variety in marinade recipes yields great variability in terms of efficacy of HAA inhibition or promotion. While marinating chicken did reduce PhIP significantly, this effect was offset by increases in other HAA. The sum of the 5 HAA measured in the analysis of these samples indicates that marinated chicken is not significantly lower in HAA overall than non-marinated chicken. Based on these results, there is no clear, unqualified response to the question of whether marinades are generally beneficial.

The mechanism of the marinade effect on HAA formation is still not understood. Evidence from model systems showed that sugar can either inhibit or promote HAA formation in cooked meats depending on its ratio to the creatine or amino acid content of the meat (Skog and Jägerstad 1991). About 12% of the marinades in the present study included sugar as an ingredient; other ingredients in many of the marinades, such as oyster sauce, ginger, garlic, or wine, include natural sugars. A more detailed investigation of individual marinade recipes might show correlations between sugar content and HAA concentrations and might explain the increases in the imidazoquinoxaline concentrations.

Marinated chicken lost significantly less weight than non-marinated chicken, while cooking time and temperature were not significantly different, suggesting that the application of a marinade may prevent water loss during cooking. In a study of PhIP formation in chicken cooked by several different methods, Persson et al. observed that cooking temperature and a parameter that they called "rate of drip loss" had a direct, positive impact on PhIP formation. Rate of drip loss, as they defined it, requires knowledge of the internal temperature of the meat at the center, a measurement not taken during the cooking of these Singapore samples, but, according to those authors, is more predictive of PhIP formation than is weight loss due to cooking (Persson et al. 2002). Jägerstad et al. suggested that water may act as a temperature regulator, in addition to serving as a solvent for HAA precursors, and, hence, may affect HAA formation (Jägerstad and Skog 1991; Jägerstad et al. 1991). The retention (or addition) of water through marinating might either inhibit the formation of HAA while cooking, or may effectively dilute the HAA that form in a given mass of meat. Salt, which was included in about 41% of the marinades, was shown to increase water retention when mixed into ground beef before frying, thereby affecting HAA formation possibly by altering meat internal or surface temperatures or by affecting the transport of HAA precursors within the meat (Persson et al. 2003). Whether or not salt in a marinade coating the chicken surface would have the same effect is an important question. The observed reduction in PhIP in the marinated chicken may be explained in part by retention of water near the meat surface, and the efficacy of the marinade effect could relate to the individual marinade ingredients or even to the viscosity of the marinade.

This protective effect of water content may also explain three observations regarding fish and HAA concentration in this study. First, fish lost significantly less weight due to cooking

than either type of chicken, even though it cooked for a similar length of time. While the raw weight of the non-marinated chicken samples, 293 ± 23.5g (mean ± standard deviation, n=41), did not differ significantly from the raw weight of the marinated chicken samples, 287 ± 28.0g (n=38), the raw fish samples were significantly higher in weight, 423 ± 63.7g (n=37), than the chicken samples of either type ($p<0.001$). Still, no significant difference in length of cooking time was observed among any of the meat types. Second, fish was significantly lower in PhIP and 4,8-DiMeIQx, as well as total HAA than non-marinated chicken. Fish was also significantly lower in MeIQx and both isomers of DiMeIQx than marinated chicken, though it was not significantly lower in terms of total HAA. Some of the marinades used may have promoted the formation of the imidazoquinoxaline compounds in chicken, perhaps due to the inclusion of certain ingredients. Since fish was quite similar to marinated chicken in terms of PhIP and IFP concentration, this again suggests the possibility of a common mechanism involving the natural water content in fish, with its skin as a natural barrier to water loss, and the protective effect of the marinade on water content in chicken. The marinade coating the complete surface of the chicken samples in that treatment group might have acted in much the same way as the skin on the fish in hydrating and protecting the meat from water loss, and the different marinades might have protected the chicken from HAA formation to greater or lesser extents depending on variations in their viscosities. Third, the cooking surface temperature was significantly higher for fish than for either type of chicken, and one might have predicted that HAA concentration would be higher as a consequence; however, just the opposite was observed. This seeming paradox was also seen in the low HAA concentrations reported in a survey of “fast-food” products, which are cooked at high temperatures, but for a relatively short cooking time (Knize et al. 1995), a phenomenon

similar to that noted here in fish. Moreover, higher water content in fish could mitigate the effects of a higher surface temperature and inhibit HAA concentration. Reported values of natural water content in raw fish, ranging from 67-77% in one study (Burger et al. 2005) are only slightly higher than that reported for raw chicken thigh, 66% (FSIS 2000), but this difference, coupled with the barrier to water loss that the fish skin provided, or perhaps greater water holding capacity of the fish muscle tissue itself, might account for some of these observed differences between fish and chicken. Chiu et al. reported lower HAA and decreased weight loss in chicken legs cooked with the skin compared to chicken legs cooked without the skin, and they speculated that the effect of the skin on water holding capacity could explain their observations (Chiu et al. 1998). Basic differences in the composition of different meat types and their relationships to HAA formation have been investigated through model systems as well as with actual meats (Jägerstad and Skog 1991; Pais et al. 1999). It is possible that these differences in creatine and free amino acids, rather than water content, may explain observed differences in HAA concentration between fish and chicken.

The various interactions among many cooking conditions need further investigating, and carefully controlled experiments would help to answer some of the questions that this work presents. Further work on the mechanism of the marinade effect needs to be done before marinating can be recommended as a means of reducing HAA formation: two possibilities for further work are experiments in which individual marinade ingredients and in various combinations of ingredients are tested, and also studies in which physical characteristics of the marinade such as viscosity are varied. Additional experimental work should also be done in order to understand the relationship between HAA formation in cooked meats and such

parameters as physical characteristics of the muscle tissue, water content, and water holding capacity in meats.

This survey of Singapore Chinese meats indicates that this population is exposed to HAA in their daily diet at levels comparable to estimates at the lower end of the range of HAA values determined in other countries; provides a realistic picture of the variety of effects on HAA formation of marinating chicken before frying and of the differences between chicken and fish; and raises interesting questions that need addressing in carefully controlled studies. The information from this study and future experiments, along with dietary intake information such as that recently obtained in Singapore (Koh et al. 2005) (Wong et al. submitted), will help to build a valuable database for researchers interested in the connection between diet and cancer.

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