JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Effect of Red Wine Marinades on the Formation of Heterocyclic Amines in Fried Chicken Breast

ROSA BUSQUETS,† LLUÍS PUIGNOU,*,† MARIA TERESA GALCERAN,† AND KERSTIN SKOG§

Departament de Química Analítica, Facultat de Química, Universitat de Barcelona, Martí i Franquès 1-11, E-08028 Barcelona, Spain, and Department of Food Technology, Engineering and Nutrition, Lund University, P.O. Box 124, SE-221 00 Lund, Sweden

Genotoxic heterocyclic amines (HAs) are formed via the Maillard reaction and free radical reaction mechanisms when meat or fish is cooked at usual cooking conditions. In this paper, the effect of the addition of red wine was tested to study if it interferes in HA formation. Fried chicken breast was the food item chosen, and three different red wines, characterized in terms of grape varieties, free amino acids, antioxidant properties, and metallic composition, were used to marinate meat prior to the heating process. Unmarinated samples and samples marinated with an ethanol−water mixture provided reference HA levels. The frying experiments were performed under well-controlled temperature and time conditions. The samples were analyzed for HA content using solid-phase extraction and LC-MS/MS. DMIP, PhIP, MeIQx, 4,8-DiMeIQx, IFP, TMIP, harman, and norharman were identified in fried chicken breast. Red wine marinades were found to reduce the formation of some of the HAs formed. PhIP, with a reduction of up to 88%, was the most minimized amine, although the formation of harman was enhanced.

KEYWORDS: Heterocyclic amines; fried chicken; wine marinades

INTRODUCTION

During heating of food products, the Maillard reaction causes changes in the taste, flavor, color, and nutritional value with respect to the raw food. However, the temperatures reached in some cooking practices favor reactions between compounds inherent in meat and fish, yielding harmful compounds. Heterocyclic aromatic amines (HAs) are an example of mutagenic species that are formed at ppb levels when meat and fish are heated at normal household conditions (*1*−*5*).

Several HAs have been found to be carcinogenic in long-term animal experiments (*6*−*8*), and their ingestion has been frequently linked with increased risk of several types of human cancer (*9*−*14*), although other studies have not shown any association with certain types of cancer (*15*−*17*). IARC (*18*) classified some HAs as probable or possible human carcinogens and recommended a decrease in their intake. Because the complete avoidance of HAs is impractical, the prevention of their formation seems to be the best approach. The keys to reduce daily life exposure to HAs are the identification of cooking practices that minimize their generation and the communication of the health benefits of these cooking practices to the consumers. Moreover, the suggested cooking methods should confer good taste and appetizing appearance to the food and should be simple enough to be easily adopted by the general public.

Creatine, free amino acids, and monosaccharides were shown to form HAs via the Maillard reaction (*19*−*23*). Free radical Maillard intermediates were also proposed to be involved in HA formation (*24*−*27*). Temperature, time, transport of water, and water-soluble substances are physical parameters with important effects on the amount of generated HAs. The formation of HAs may be significantly reduced by decreasing cooking time and temperature and diminishing the drip loss by the addition of ingredients with water-holding capacity (*28*, *34*). Among the ingredients that can chemically reduce the yield of HAs, there are those that can compete with HA precursors such as certain low molecular weight carbohydrates (*35*). The addition of natural products containing antioxidants that may act as free radical scavengers, such as polyphenols, reduces the mutagenic activity of the cooked products and the amount of HAs in the heat-processed meat. Among these additives, the effect of cherry tissue (*36*), tea (*37*, *38*), olive oil (*39*, *40*), onion (*41*, *42*), tomato (*43*), garlic (*41*, *44*−*46*), rosemary, thyme, sage, and brine (*44*, *47*) has been demonstrated. Earlier works studied the effect of marinating using mixtures of culinary ingredients, and a change in the generation of some HAs was observed (*45*, *48*−*50*).

The aim of this work was to contribute to the search for cooking practices that could minimize the risk of exposure to HAs. Marinating with red wine is a cooking practice used in several countries, for example, in Spain. The effect of marinating

* Corresponding author (fax +34 93 402 12 33; telephone +34 93 402 12 86; e-mail lluis.puignou@ub.edu).
† Universitat de Barcelona.
§ Lund University.

10.1021/jf0616311 CCC: $33.50
Published on Web 09/16/2006

in red wine with different contents of antioxidants on the formation of HAs in fried chicken was evaluated

MATERIALS AND METHODS

Chemicals. All chemicals and solvents were of HPLC or analytical grade. 2-Amino-1,6-dimethylimidazo[4,5-*b*]pyridine (DMIP), 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), 2-amino-3-methylimidazo[4,5-*f*]quinoxaline (IQx), 2-amino-3,4-dimethylimidazo[4,5-*f*] quinoline (MeIQ), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (4,8-DiMeIQx), 2-amino-3,7,8-trimethylimidazo[4,5-*f*]quinoxaline (7,8-DiMeIQx), 2-amino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole (Glu-P-1), 2-aminodipyrido-[1,2-*a*:3′,2′-*d*]imidazole (Glu-P-2), 2-amino-3,4,7,8-tetramethylimidazo-[4,5-*f*]quinoxaline (TriMeIQx), 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-1), 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-2), 2-amino-9*H*-pyrido-[2,3-*b*]indole (AαC), and 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole (MeAαC) were purchased from Toronto Research Chemicals (Toronto, Canada). 1-Methyl-9*H*-pyrido-[4,3-*b*]indole (harman) and 9*H*-pyrido-[3,4-*b*]indole (norharman) were purchased from Aldrich (Steinheim, Germany). The chemical purity of the compounds was >99%, according to the manufacturers. 2-Amino-1,6-dimethylfuro[3,2-*e*]-imidazo[4,5-*b*]pyridine (IFP) was kindly provided by Mark G. Knize, Lawrence Livermore National Laboratories, Livermore, CA, and 2-amino-1,5,6-trimethylimidazo[4,5-*b*]pyridine (TMIP) was bought from the NCI Chemical Carcinogen Reference Standard Repository (Kansas City, MO). Stock standard solutions of 150 μg/g in methanol were prepared and used for further dilution. A mixture of the different HAs in MeOH (2 μg/g) was used as spiking mixture. TriMeIQx was used as internal standard.

Materials for solid-phase extraction were diatomaceous earth (Isolute), obtained from Sorbent AB (Västra Frölunda, Sweden), and PRS and C_{18} columns (Varian) from Scantech Lab (Partille, Sweden).

Chemicals for the determination of wines' antioxidant properties were Folin−Ciocalteu's reagent, purchased from Panreac (Barcelona, Spain), gallic acid from Merck (Darmstadt, Germany), and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), potassium persulfate, and 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt [ABTS-$(NH_4)_2$] from Sigma-Aldrich (Steinheim, Germany).

Stock standard solutions for the screening of metals in wines were obtained from High Purity Standards (Charleston, SC). These stock solutions had a concentration of 1 g/L traceable to The National Institute of Standards and Technology (Gaithersburg, MD). Standards were prepared by diluting the stock solutions with 1% nitric acid in aqueous media.

Wines. Three different red wines (A, B, and C) were used to study the effect of marinating meat with wine. All of them were from the wine cellar called "Mas dels Frares" that belongs to the Enology Faculty of "Universitat Rovira i Virgili" (Tarragona, Spain). Grape varieties used in winemaking have a high influence on the flavors and composition of wine. Wine A was a breeding wine, made of Cabernet Sauvignon (50%), Merlot (20%), Tempranillo (15%), and Carinyena (15%), vintage 2001. Wine B was a superior wine made of Cabernet Sauvignon (50%), Merlot (25%), and Syrah (25%), vintage 2001. Wine C was a superior wine made of Cabernet Sauvignon (33%), Merlot (17%), Tempranillo (33%), and Syrah (17%), vintage 2000.

Antioxidant Properties. Total antioxidant activity and total phenolic content were determined in the three red wines. Both analyses were based on spectrophotometric determinations using a Unicam UV−vis model 4-100 spectrophotometer (Thermo Elemental, Madrid, Spain). The total antioxidant activity was determined with the method called Trolox equivalent antioxidant capacity (TEAC) (*51*). Measurements were carried out at a wavelength of 734 nm at 30 °C. Reacting time was fixed at 5 min.

Total phenolic content was determined using the Folin−Ciocalteu spectrophotometric method at 750 nm (*52*, *53*). Gallic acid was used as standard for the calibration curve. Both assays were made in triplicate.

Amino Acids and Metals. Free amino acids were determined in the wine samples by ion-exchange chromatography using a Biochrome30 Amino Acid Analyzer (Biochrome, Cambridge, U.K.) as described by Arvidsson et al. (*54*).

Screening of metals in the wine samples was performed using inductively coupled plasma mass spectrometry (ICP-MS) and inductively coupled plasma optical emission spectroscopy (ICP-OES). ICP-MS system was a Perkin-Elmer Elan 6000 (Boston, MA) working at 1150 W (forward power); nebulizer gas flow was 0.55 L/min and sample flow was 1 mL/min. The quantitation was carried out by external calibration using Rh at 10 μg/L as internal standard. The ICP-OEP system was a Perkin-Elmer Optima 3200RL working at 1150 W (forward power); nebulizer gas flow as 0.8 L/min, auxiliary gas flow was 0.5 L/min, and plasma gas flow was 15 L/min. Samples were diluted from 20 to 50 times for the analysis.

Meat Samples and Cooking. Fresh chicken breasts were purchased from a local store. Each chicken breast provided one chicken fillet with size of 6 × 7 cm, 1 cm in thickness, and a weight of 46 g. For each condition studied, three chicken fillets were marinated and cooked independently. Finally, the crust from fillets was removed from the moist meat interior and was freeze-dried. For the analysis, the freeze-dried crusts were blended and constituted a combined sample representative of the three independent cooking processes.

Preliminary studies were carried out to select the cooking conditions that provided the most appetizing fried samples. Different cooking temperatures and cooking times, meat shape (fillet, ground meat patty), and use of fat (margarine) were tested. All samples were marinated in water for 15 min prior to cooking. The samples were fried in a Teflon-coated frying pan.

Marinating. To marinate, one chicken fillet (46 g) and wine (70 mL) were placed inside plastic bags. Bags were tied in a knot after pushing air out. Marinating was carried out at room temperature for 30 min, 3 h, and 24 h because both short and long marinating times are normal cooking practices. In addition, samples were marinated in water or ethanol/water (1:7).

Frying. A thermostat-controlled frying pan, 235 × 235 mm, was used for the experiments. The samples were put in the pan when the pan temperature had reached 220 °C. The samples were fried without fat for 5 min/side and were turned once during frying. Five K-type thermocouples, connected to a data logger, were used to monitor the temperatures during frying; two thermocouples were inserted in the center of one fillet, two were fixed ∼1 mm below the upper and the lower surfaces, and one thermocouple was placed between the pan surface and the fillet to measure the frying temperature. The temperature was recorded every 10 s. After frying, the crust (1−2 mm of the surface) of the fried chicken fillets was separated from the moist meat interior and freeze-dried.

Moisture Analysis and Cooking Loss during Frying. The moisture content was determined in both the crust and the moist meat interior in duplicate by gravimetric determination drying at 105 °C. Cooking loss was measured as the weight difference between the samples before and after frying.

Analysis of HAs. Each sample (2 g of freeze-dried blended crust) was homogenized with an Ultra-Turrax T 25 basic (IKA, Staufen) in 1 M NaOH and purified with the solid-phase extraction method developed by Toribio et al. (*55*) using ethyl acetate as extraction solvent. Extraction recovery rates were determined by the addition of 100 μL of spiking mixture with HA concentrations ∼2 μg/g to one sample extracted in parallel to four unspiked samples. Recoveries were evaluated for each batch of extractions. The concentrations of HAs were corrected for incomplete recovery. HAs were quantified in duplicate from fried chicken samples. The LC-MS/MS in product ion scan as described previously by Barceló-Barrachina et al. (*56*) with minor modifications was used for HA quantification. Liquid chromatography was performed with a Symmetry C_8 5 μm (2.1 mm × 150 mm) column from Waters (Milford, MA), and the mobile phase was a gradient with acetonitrile (solvent A) and 30 mM acetic acid/ammonium acetate buffer adjusted at pH 4.5 (solvent B) at a flow rate of 0.3 mL/min. The gradient separation program was as follows: 0−0.5 min, 5% A; 0.5−15 min, 5−20% A; 15−18 min, 20−60% A; 18−24 min, 60% A; 24−27 min, return to initial conditions; 5 min postrun delay. The sample volume injected was 5 μL. At these conditions, the response was linear up to 1 μg/g. The chromatograph was a Spectra Physics P2000 and the mass spectrometer an ion trap LCQDeca equipped with an electrospray ionization source, both from Thermo Finnigan (San Jose, CA). The chromatogram was segmented in three windows, and full scan and several product ion scans were monitored. This acquisition

Table 1. MS and MS/MS Parameters

segment	time (min)	analyte	mode	scan range *m/z*	NCE[a] (%)	precursor ion $[M + H]^+$ *m/z*	product ions used for quantitation m/z	product ions used for quantitation tentative assignation
1	0–14.2		full scan	150–280				
	0–14.2	DMIP	product ion scan	140–170	41	163	148	$[M + H - CH_3]^{\bullet+}$
							146	$[M + H - NH_3]^+$
	0–14.2	IQx	product ion scan	150–206	44	200	185	$[M + H - CH_3]^{\bullet+}$
	0–14.2	MeIQx	product ion scan	165–220	46	214	199	$[M + H - CH_3]^{\bullet+}$
							187	$[M + H - HCN]^+$
							173	$[M + H - C_2NH_3]^+$
2	14.2–19.7		full scan	150–280				
	14.2–19.7	7,8-DiMeIQx	product ion scan	180–235	45	228	213	$[M + H - CH_3]^{\bullet+}$
							187	$[M + H - C_2NH_3]^+$
	14.2–19.7	4,8-DiMeIQx	product ion scan	180–235	45	228	213	$[M + H - CH_3]^{\bullet+}$
							187	$[M + H - C_2NH_3]^+$
	14.2– 19.7	norharman	product ion scan	110–175	49	169	168	$[M + H - H]^{\bullet+}$
							142	$[M + H - HCN]^+$
							115	$[M + H - 2HCN]^+$
	14.2–19.7	TriMeIQx	product ion scan	195–250	47	242	227	$[M + H - CH_3]^{\bullet+}$
							201	$[M + H - C_2NH_3]^+$
	14.2–19.7	harman	product ion scan	110–190	50	183	182	$[M + H - H]^{\bullet+}$
							168	$[M + H - CH_3]^{\bullet+}$
							156	$[M + H - HCN]^+$
							115	$[M + H - C_3H_4N_2]^+$
3	19.7–23		full scan	150–280				
	19.7–23	PhIP	product ion scan	200–230	49	225	210	$[M + H - CH_3]^{\bullet+}$
							208	$[M + H - NH_3]^+$

[a] Normalized collision energy.

Table 2. Concentration of Amino Acids in the Studied Wines

amino acid	concentration (μmol/L) wine A	wine B	wine C
aspartic acid	129	55	193
threonine	73	104	100
serine	95	139	126
asparagine	49	30	3
glutamic acid	252	368	289
glutamine	39	51	75
proline	11686	15646	6904
glycine	213	290	236
alanine	456	854	558
valine	112	167	170
cysteine	20	4	22
methionine	25	13	32
isoleucine	45	49	72
leucine	108	113	183
tyrosine	31	17	99
phenylalanine	69	84	132
lysine	146	181	182
histidine	15	18	45
arginine	39	39	131
tryptophan			

Table 3. Concentration of Metals in the Studied Wines

element	concentration (mg/L) wine A	wine B	wine C
Li[a]	0.01	0.01	0.01
Na[b]	17.5	21.5	13.2
K[b]	1188.6	1369.2	1155.7
Rb[a]	0.7	0.6	0.5
Cs[a]	0.01	<0.002	<0.002
Mg[b]	84.3	100.8	101.1
Ca[b]	70.9	49.1	63.3
Sr[b]	1.0	1.1	1.0
Ba[a]	0.1	0.1	0.1
Cr[a]	0.1	0.09	0.07
Mo[a]	<0.004	<0.004	<0.004
Mn[a]	0.8	0.8	0.8
Fe[b]	1.7	0.9	0.9
Ni[a]	0.02	0.02	0.02
Cu[a]	0.5	0.01	0.02
Zn[a]	0.4	0.5	0.8
B[a]	7.5	9.2	8.6
Al[a]	0.3	0.3	0.5
Si[b]	12.5	14.6	19.2
Pb[a]	0.01	0.01	0.01
Sb[a]	0.01	0.02	0.02
S[b]	366.4	196.4	202.6

[a] ICP-MS. [b] ICP-OES.

mode allowed quantification of known compounds and identification of unknowns. MS/MS parameters are given in **Table 1**.

RESULTS

Characteristics of the Wines. The different compositions of the wines used in the marinades can affect the yield of HAs; thus, the wine samples were characterized in terms of antioxidant properties and amino acid and metal compositions because these parameters may have a role in HA formation.

Total phenol contents and antioxidant capacities were different for the three wines. The average concentrations of phenols (gallic acid equivalents) in wines A, B, and C were 2660, 3480, and 4410 mg/L, respectively. The antioxidant capacities for wines A, B, and C were 35, 42, and 47 mM Trolox equivalents, respectively.

A wide range of free amino acid concentrations, from undetectable to 16 mM, were found in the wines (**Table 2**). Proline, with concentrations ~7−16 mM, was the most abundant amino acid. These concentrations of free amino acids are in accordance with those reported in the literature (*57*).

The concentrations of metals, sulfur, and boron in the wines are given in **Table 3**. The concentrations of these elements were similar in all of the wines, with the exception of copper, which was found at 20 and 50 times higher concentrations in wine A than in wines C and B, respectively.

Reductant sugars analysis, based on the Fehling method, provided relatively low and very similar values for all of the

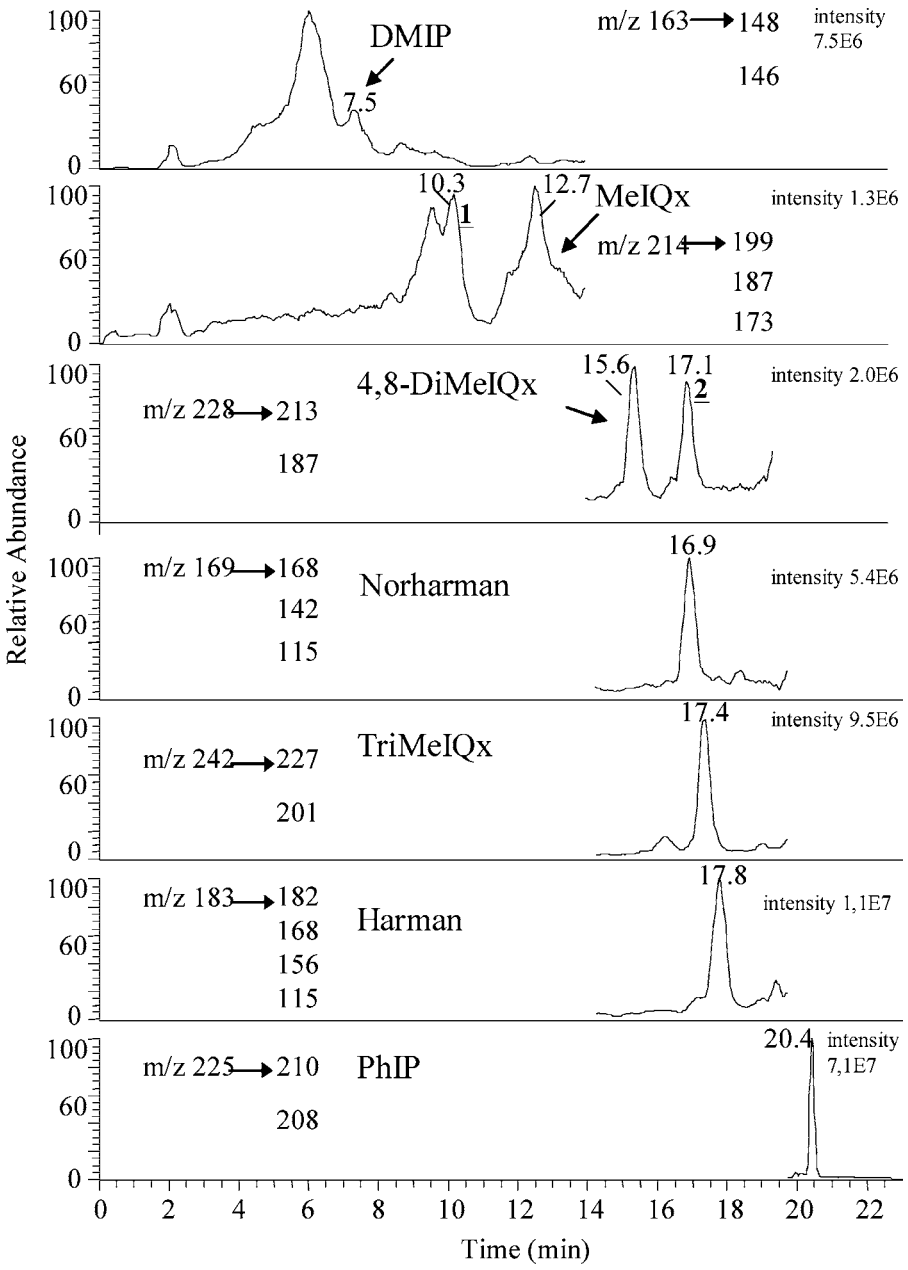

Figure 1. Product ion scan chromatograms of heterocyclic amines in a fried chicken fillet without previous marination.

wines (11−17 mol/mL). The acidities of the wines, expressed as the pH, were found to be very similar (3.5−3.7) in all cases.

Concentration of HAs. HAs were analyzed in unmarinated and marinated fried chicken. DMIP, PhIP, MeIQx, 4,8-DiMeIQx, norharman, and harman were identified in all samples at concentrations above the limit of quantification (S/N = 10) using product ion scan mode. However, DMIP eluted in the tail of a coeluting peak and was not quantified. IFP and TMIP were identified, using full-scan mode, at concentrations lower than the limit of quantification. The concentrations of HAs in the fried unmarinated chicken samples, expressed on a cooked fillet basis, were as follows: PhIP, 72 ± 9 ng/g; MeIQx, 2.3 ± 0.3 ng/g; 4,8-DiMeIQx, 2.3 ± 0.03 ng/g; norharman, 19 ± 2 ng/g; and harman, 6.4 ± 0.2 ng/g. Inaccuracies in MeIQx determination could exist due to the non-Gaussian form of the chromatographic peak. The recoveries of HAs were evaluated for each batch of extractions and were used to correct the concentration values in the samples. To set the standard addition calibration curve within the linearity range of the method, the quantification of the most concentrated HAs required a dilution of the purified samples, initially reconstituted in 0.1 mL. Then, for unmarinated samples, ethanol/water-marinated samples, and 30 min wine marinated samples, PhIP required a dilution (1/6) of the purified extracts. Besides, it was necessary to use the same dilution for PhIP quantification when using wine B in the 3 h marinade. Norharman and harman required a dilution (1/6) of the purified samples when wine C was used for marinating. In addition, harman was diluted (1/6) when marinating was performed with wine A for 3 and 24 h. In the other marinating conditions, HAs were determined in purified extracts from 2 g of crust reconstituted in 0.1 mL without any additional dilution. The average recovery rates were 35, 48, 46, 48, and 40% for MeIQx, 4,8-DiMeIQx, PhIP, norharman, and Harman, respectively.

Figure 1 shows chromatograms of the most abundant HAs found in an unmarinated fried chicken fillet. Interestingly, in the chromatograms for MeIQx and DiMeIQx, two peaks (denoted peaks 1 and 2) appeared at retention times of 10.3 and 17.1 min, respectively. Their product ion mass spectra matched those found by Turesky et al., who tentatively assigned

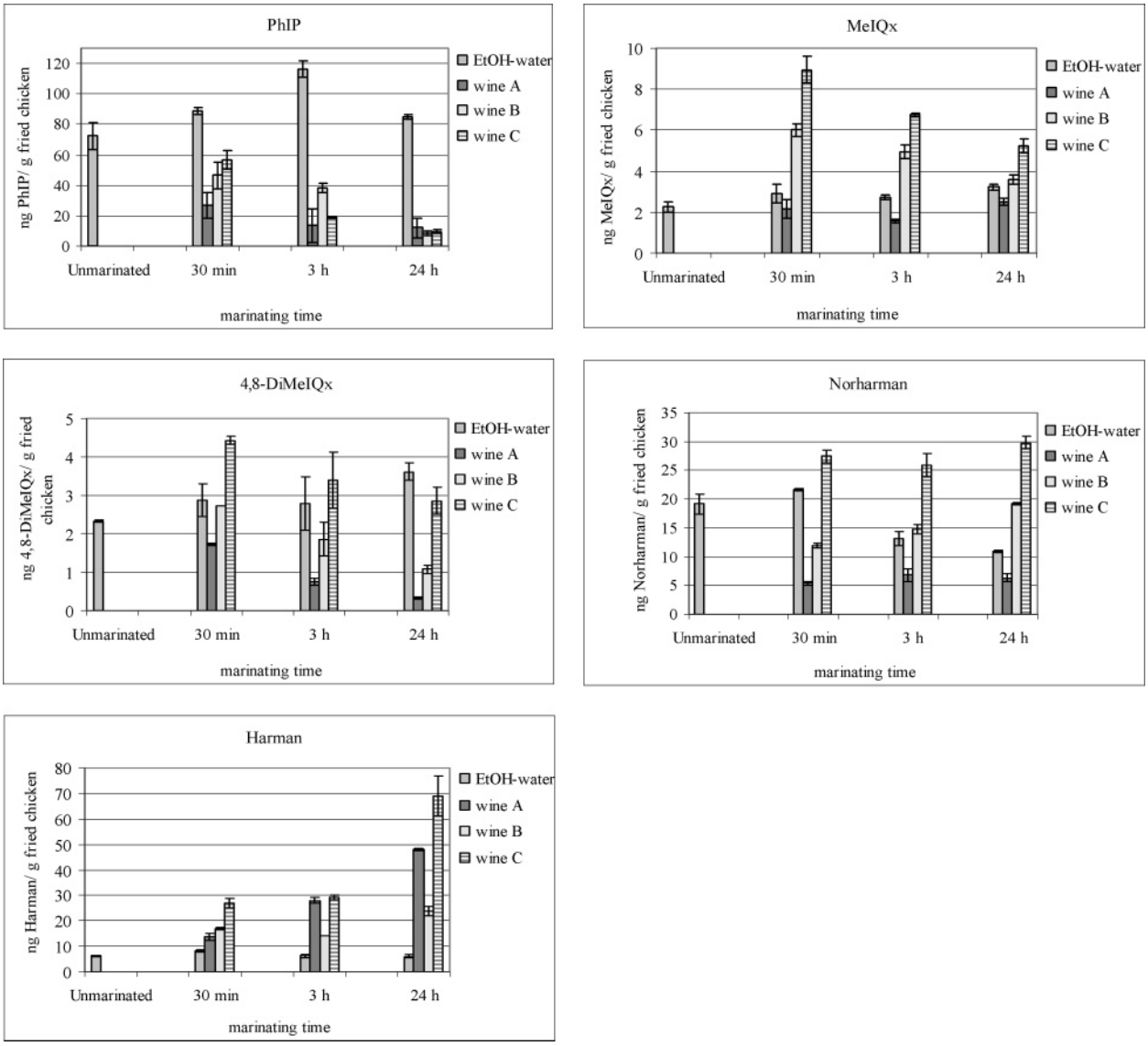

Figure 2. Effect of marinating media and marinating time on HA formation.

them to a linear tricyclic ring of 8-MeIQx and to an isomer of DiMeIQx, respectively (*58*).

Figure 2 displays the concentrations of HAs formed in the unmarinated and different marinated fried chicken breasts. Error bars indicate the standard deviation obtained in the determination of HAs. Compared with the unmarinated samples, marinating with ethanol/water (1:7) resulted in somewhat increased levels of HAs except for harman and norharman at 3 and 24 h of marinating. These data show the effect of the liquid media on the formation of HAs and provide reference values for the amounts of HAs.

All wine marinades reduced significantly the amount of PhIP formed (**Figure 2**), for instance, up to 88% when marinating for 24 h with wine B. In contrast, wines B and C enhanced the formation of MeIQx, especially at the shorter marinating times. Wine A reduced the formation of 4,8-DiMeIQx, whereas wine C enhanced the formation. Wine A also reduced the formation of norharman, whereas wine C enhanced the formation. Conversely, all of the wines tested enhanced the formation of harman especially when marinating was carried out for long periods (**Figure 2**).

Moisture and Cooking Loss. The moisture content varied between 38−50% in the crust and 50−58% in the moist meat interior. The moisture content in the raw meat was 75%. Average cooking losses of around 50, 52−55, and 55−59% were observed for unmarinated samples, marinated samples, and samples marinated in ethanol/water, respectively.

DISCUSSION

On the basis of preliminary studies, it was decided to marinate and fry chicken fillets without fat for 5 min/side at 220 °C, which provided medium-toasted samples with good organoleptic properties. Fried chicken was the food item selected because it is the type of food in which larger amounts of HAs are usually generated; therefore, it would be easier to observe the effect of wine marinades on HA formation. Fillet, instead of ground meat patty, was the meat shape chosen because the ground meat patty's structure became too fragile in marinades. Because of possible influence of fat on HA formation, and to simplify the experiments and the interpretation of the results, fat was not used for cooking.

Three wines with different compositional profiles were used to marinate chicken breasts to study the effect on HA formation. To distinguish between the physical and chemical effects due to the marinating media, meat samples were also marinated prior to cooking in an ethanol/water mixture, with the same alcoholic composition as the wines. These samples were considered as

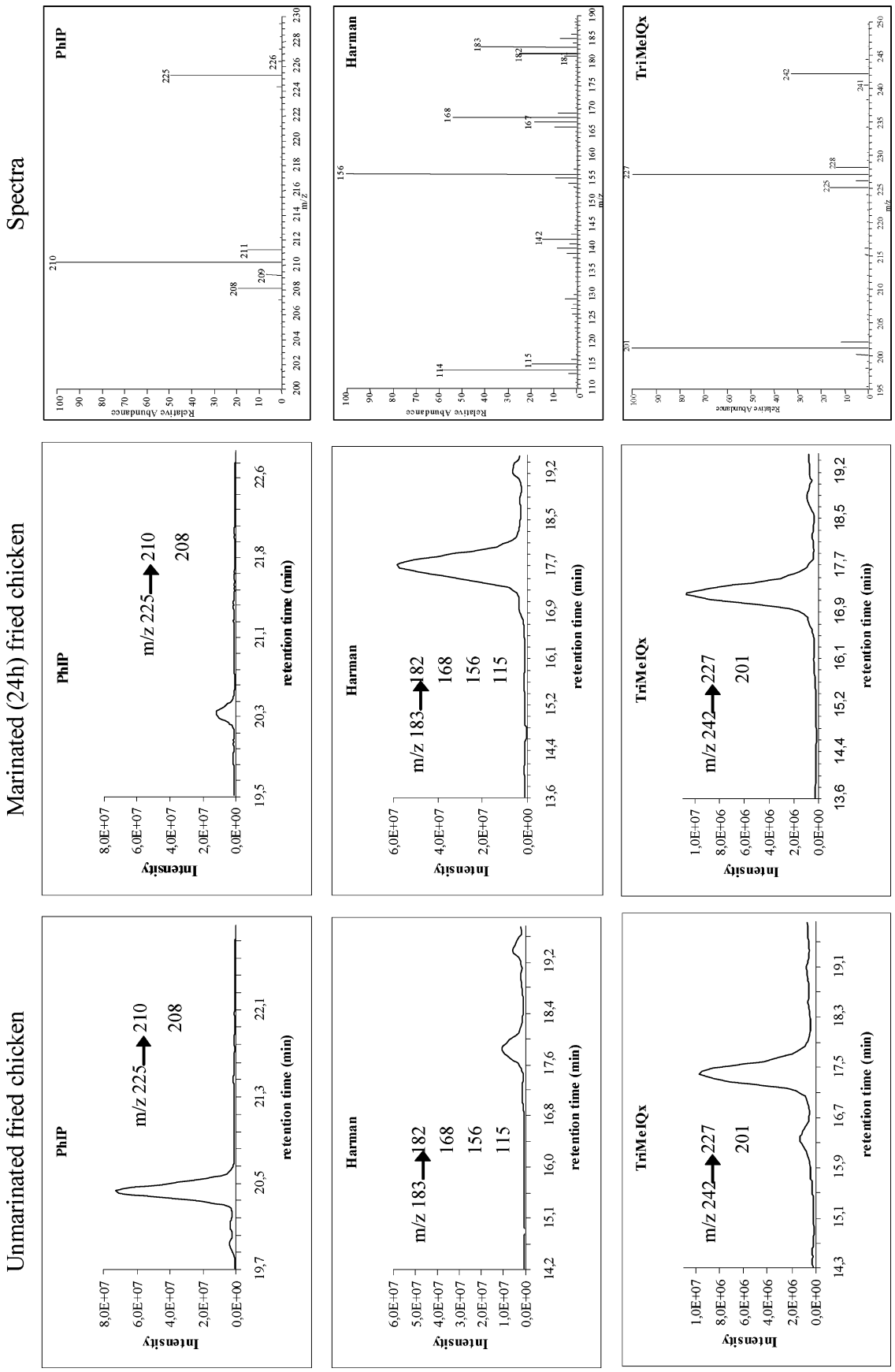

Figure 3. Effect of a 24 h marinade with wine B on the formation of PhIP and Harman in fried chicken. The corresponding chromatograms of the internal standard, TriMeIQx, at each condition are also shown. In addition, the spectra of the studied compounds are provided. The chromatograms shown correspond to the sum of the product ions.

reference or control samples in which the HA content was affected only by the liquid media. In addition, unmarinated fried chicken breasts were analyzed.

The occurrence of HAs in unmarinated fried chicken breast are in agreement with results obtained by Solyakov et al., who studied different cooking times and temperatures (*59*); however, in the present study 4,8-DiMeIQx, PhIP, and norharman were found at higher concentrations (5, 2, and 5 times, respectively). In addition, we detected the pyridnes IFP and TMIP in the unmarinated chicken breast, but the signal-to-noise ratio was too low to allow accurate quantification. In other studies of fried meat, the levels of IFP and TMIP ranged between nondetectable and 46 ng/g for IFP and 3 ng/g for TMIP (*5*, *50*, *60*).

The reducing effect of wine marinating on the formation of PhIP, one of the most abundant HAs in poultry products (*1*), was very important. All three wines tested reduced the PhIP content, from 72 ng/g in unmarinated fried chicken to 8−12 ng /g in marinated fried chicken, that is, 83−88% decrease. A pronounced reduction of PhIP was found in another study (*49*) in which a mixture of several ingredients (olive oil, brown sugar, cider vinegar, lemon juice, crushed garlic, salt, and mustard) was used to marinate chicken before frying. A significant reduction of PhIP was observed using Singapore Chinese marinades (*50*). Polyphenols and other antioxidant compounds present in wines may play a role in PhIP formation (*38*, *61*−*63*). In our study, the reducing effect on PhIP formation may also be related to the presence of some amino acids because it has been reported that proline or tryptophan can compete in the reaction of creatinine with precursor molecules of HAs (*64*). However, only proline was found at high concentrations in all of the wines tested (**Table 2**), and possibly the meat could absorb proline from the marinating medium, resulting in a reduction of HA formation. Proline content in the wines was higher than the reported level in chicken meat (*59*). In contrast, phenylalanine, which is a precursor of PhIP, was found at lower concentrations in the wines than in chicken meat (*59*).

The effect of antioxidants on the generation of HAs has been reported to depend on the type, concentrations, and synergistic effects of pro- and antioxidants, leading to an enhancement or a reduction of HA generation. For example, Vitaglione et al. (*43*) and Johansson et al. (*65*) observed that the rate of inhibition or promotion of the quinoxalines in different model systems was not always correlated with the antioxidant concentrations. In our study, there was a significant positive correlation between the antioxidant properties of the wine and the enhancement of MeIQx and 4,8-DiMeIQx formation. The highest enhancement of quinoxalines occurred at short marinating times when using the highest antioxidant wines and was gradually reduced at longer marinating times (see **Figure 2**), probably because other compounds present in the wines may have counteracted the enhancing effect. In contrast, inhibition of 4,8-DiMeIQx, up to 87%, was achieved when marinating with the wines with lowest antioxidant properties for long marinating times. The results are in agreement with those found in another study in which MeIQx formation in grilled marinated meat increased at some conditions (*49*) and with results found in a study on model systems in which several food-derived antioxidants were heated together with a mixture of HA precursors (*63*). In this last cited work some antioxidants markedly suppressed MeIQx formation and mutagenicity, whereas some others, such as nordihydroguaiaretic acid, promoted MeIQx production. This fact shows that both anti- and pro-oxidative effects can be exhibited by antioxidants, which caused different rates of formation of HAs.

The increase of the formation of MeIQx and 4,8-DiMeIQx at certain conditions may also be related to the presence of some metal ions. Some authors have reported enhanced mutagenic activity after heating HA precursors together with iron, which has been attributed to pro-oxidant properties and to the formation of HAs via radical reactions (*66*−*68*). Therefore, a screening of metals, in addition to sulfur and boron, in the wines was performed (see **Table 3**). However, the small differences in the metal compositions of the wines, which is strongly influenced by winemaking practices, soil, climate, culture, or grape varieties, could not be correlated with the enhancement of the quinoxaline formation. Neither total sugars nor pH appeared to have any correlation with the observed occurrence of HAs in fried chicken breast because the corresponding values found in the wines were very similar to those usually present in the studied meat (*59*). As a consequence, these parameters may have minimum influence on the formation of the mutagens.

Wine C showed increased amounts of norharman at all marinating times, up to 56% when marinating was carried out for 24 h. The enhancement of norharman occurrence is well correlated with the increasing antioxidant properties of the wines as was found for the quinoxalines and could constitute evidence that HA formation involves radical intermediates (*69*). The formation of norharman could not be correlated with the presence of tryptophan, a precursor of norharman, because this amino acid was not found in the wines.

Wine marinades noticeably enhanced the formation of harman and reduced the formation of PhIP. **Figure 3** shows the effect of wine B marinade on the occurrence of harman and PhIP in fried unmarinated and marinated chicken fillets. There was a clear tendency that harman increased with longer marinating times, up to 652% (wine A), 278% (wine B), and 978% (wine C) in the 24 h marinades. However, no correlation between the antioxidant properties of the wines and the enhancement of Harman formation was found.

In conclusion, several HAs including IFP and TMIP were found in fried chicken samples, together with two not commonly reported quinoxaline-type compounds. Our data clearly show that the occurrence of HAs in fried chicken is affected by red wine marinades. A correlation between the antioxidant properties of the wine and the amount of MeIQx, 4,8-DiMeIQx, and norharman was found. For the shortest marinating time and the greater antioxidant capacity, the higher amount of these amines was formed. In contrast, long marinades with red wine caused a high inhibition of PhIP but also the highest formation of harman.

ABBREVIATIONS USED

HAs, heterocyclic amines; LC, liquid chromatography; MS, mass spectrometry; DMIP, 2-amino-1,6-dimethylimidazo[4,5-*b*]pyridine; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]-pyridine; MeIQx, 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; 4,8-DiMeIQx, 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline; IFP, 2-amino-1,6-dimethylfuro[3,2-*e*]-imidazo[4,5-*b*]-pyridine; TMIP, 2-amino-1,5,6-trimethylimidazo[4,5-*b*]pyridine; harman, 1-methyl-9*H*-pyrido-[4,3-*b*]indole; norharman, 9*H*-pyrido-[3,4-*b*]indole; IQ, 2-amino-3-methylimidazo[4,5-*f*]quinoline; IQx, 2-amino-3-methylimidazo[4,5-*f*]quinoxaline; MeIQ, 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline; 7,8-DiMeIQx, 2-amino-3,7,8-trimethylimidazo[4,5-*f*]quinoxaline; Glu-P-1, 2-amino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole; Glu-P-2, 2-aminodipyrido[1,2-*a*:3′,2′-*d*]imidazole; TriMeIQx, 2-amino-3,4,7,8-tetramethylimidazo[4,5-*f*]quinoxaline; Trp-P-1, 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole; Trp-P-2, 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole; AαC, 2-amino-9*H*-pyrido-[2,3-*b*]indole, MeAαC, 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole; ppb, parts per billion; IARC, International Agency for Research on Cancer; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; TEAC, Trolox equivalent antioxidant capacity; ABTS, 2,2′-

azinobis(3-ethylbenzothiazoline-6-sulfonic acid); ICP, inductively coupled plasma; OES, optical emission spectroscopy.

ACKNOWLEDGMENT

We are very grateful to Ingegerd Sjöholm from Lund University for her help in the frying experiments and to Antoni Padró, from SCT of the University of Barcelona, for the performance of metals analysis. The gifts of wine samples from Josep Guasch from the Enology Faculty of Tarragona (Rovira i Virgili University) and IFP from Mark Knize from Lawrence Livermore National Laboratories are gratefully acknowledged. R.B. acknowledges Lund University and the University of Barcelona for making her stay at the department of Food Technology, Engineering and Nutrition possible.

LITERATURE CITED

(1) Skog, K.; Solyakov, A. Heterocyclic amines in poultry products: a literature review. *Food Chem. Toxicol.* **2002**, *40*, 1213−1221.

(2) Skog, K.; Johansson, M. A. E.; Jägerstad, M. I. Carcinogenic heterocyclic amines in model systems and cooked foods—a review on formation, occurrence and intake. *Food Chem. Toxicol.* **1998**, *36*, 879−896.

(3) Warzecha, L.; Janoszka, B.; Blaszczyk, U.; Strozyk, M.; Bodzek, D.; Dobosz, C. Determination of heterocyclic aromatic amines (HAs) content in samples of household-prepared meat dishes. *J. Chromatogr. B* **2004**, *802*, 95−106.

(4) Busquets, R.; Bordas, M.; Toribio, F.; Puignou, L.; Galceran, M. T. Occurrence of heterocyclic amines in several home-cooked meat dishes of the Spanish diet. *J. Chromatogr. B* **2004**, *802*, 79−86.

(5) Pais, P.; Salmon, C. P.; Knize, M. G.; Felton, J. S. Formation of mutagenic/carcinogenic heterocyclic amines in dry-heated model systems, meats, and meat drippings. *J. Agric. Food Chem.* **1999**, *47*, 1098−1108.

(6) Adamson, R. H.; Snyderwine, E. G.; Thorgeirsson, U. P.; Schut, H. A.; Turesky, R. J.; Thorgeirsson, S. S.; Takayama, S.; Sugimura, T. Metabolic processing and carcinogenicity of heterocyclic amines in nonhuman primates. *Princess Takamatsu Symp.* **1990**, *21*, 289−301.

(7) Ohgaki, H.; Takayama, S.; Sugimura, T. Carcinogenicities of heterocyclic amines in cooked foods. *Mutat. Res.* **1991**, *259*, 399−410.

(8) Wakabayashi, K.; Sugimura, T. Heterocyclic amines formed in the diet: carcinogenicity and its modulation by dietary factors. *J. Nutr. Biochem.* **1998**, *9*, 604−612.

(9) Destefani, E.; Ronco, A.; Mendilaharsu, M.; Guidobono, M.; Deneopellegrini, H. Meat intake, heterocyclic amines, and risk of breast-cancer—a case-control study in Uruguay. *Cancer Epidemiol. Biomarker Prev.* **1997**, *6*, 573−581.

(10) Sinha, R.; Chow, W. H.; Kulldorff, M.; Denobile, J.; Butler, J.; Garciaclosas, M.; Weil, R.; Hoover, R. N.; Rothman, N. Well-done, grilled red meat increases the risk of colorectal adenomas. *Cancer Res.* **1999**, *59*, 4320−4324.

(11) Sinha, R.; Kulldorff, M.; Chow, W. H.; Denobile, J.; Rothman, N. Dietary-intake of heterocyclic amines, meat-derived mutagenic activity, and risk of colorectal adenomas. *Cancer Epidemiol. Biomarker Prev.* **2001**, *10*, 559−562.

(12) Butler, L. M.; Sinha, R.; Millikan, R. C.; Martin, C. F.; Newman, B.; Gammon, M. D.; Ammerman, A. S.; Sandler, R. S. Heterocyclic amines, meat intake, and association with colon cancer in a population-based study. *Am. J. Epidemiol.* **2003**, *157*, 434−445.

(13) Terry, P. D.; Lagergren, J.; Wolk, A.; Steineck, G.; Nyren, O. Dietary intake of heterocyclic amines and cancers of the esophagus and gastric cardia. *Cancer Epidemiol. Biomarker Prev.* **2003**, *12*, 940−944.

(14) Sinha, R.; Peters, U.; Cross, A. J.; Kulldorff, M.; Weissfeld, J. L.; Pinsky, P. F.; Rothman, N.; Hayes, R. B. Meat, meat cooking methods and preservation, and risk for colorectal adenoma. *Cancer Res.* **2005**, *65*, 8034−8041.

(15) Gertig, D. M.; Hankinson, S. E.; Hough, H.; Spiegelman, D.; Colditz, G. A.; Willett, W. C.; Kelsey, K. T.; Hunter, D. J. *N*-Acetyl transferase 2 genotypes, meat intake and breast-cancer. *Int. J. Cancer* **1999**, *80*, 13−17.

(16) Destefani, E.; Ronco, A.; Brennan, P.; Boffetta, P. Meat consumption and risk of stomach-cancer in Uruguay—a case-control study. *Nutr. Cancer* **2001**, *40*, 103−107.

(17) Augustsson, K.; Skog, K.; Jägerstad, M.; Dickman, P. W.; Steineck, G. Dietary heterocyclic amines and cancer of the colon, rectum, bladder and kidney: a population-based study. *Lancet* **1999**, *353*, 703−707.

(18) International Agency for Research on Cancer (IARC). Some natural occurring substances: food items and constituents. Heterocyclic amines and mycotoxins. *IARC Monogr. Eval. Carcinog. Risks Chem. Hum.* **1993**, *56*, 163−242.

(19) Jägerstad, M.; Reuterswärd, A. L.; Öste, R.; Dahlqvist, A.; Grivas, S.; Olsson, K.; Nyhammar, T. Creatinine and maillard reaction products as precursors of mutagenic compounds formed in fried beef. *ACS Symp. Ser.* **1983**, *No. 215*, 507−519.

(20) Övervik, E.; Kleman, M.; Berg, I.; Gustafsson, J. A. Influence of creatine, amino acids, and water on the formation of the mutagenic heterocyclic amines found in cooked meat. *Carcinogenesis* **1989**, *10*, 2293−2301.

(21) Johansson, M. A. E.; Fay, L. B.; Gross, G. A.; Olsson, K.; Jägerstad, M. Influence of amino-acids on the formation of mutagenic/carcinogenic heterocyclic amines in a model system. *Carcinogenesis* **1995**, *16*, 2553−2560.

(22) Murkovic, M.; Weber, H. J.; Geiszler, S.; Frohlich, K.; Pfannhauser, W. Formation of the food associated carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) in model systems. *Food Chem.* **1999**, *65*, 233−237.

(23) Arvidsson, P.; Van Boekel, M. A. J. S.; Skog, K.; Solyakov, A.; Jägerstad, M. Formation of heterocyclic amines in a meat juice model system. *J. Food Sci.* **1999**, *64*, 216−221.

(24) Pearson, A. M.; Chen, C.; Gray, J., I; Aust, S. D. Mechanism(s) involved in meat mutagen formation and inhibition. *Free Radical Biol. Med.* **1992**, *13*, 161−167.

(25) Milic, B. L.; Djilas, S. M.; Canadanovicbrunet, J. M. Synthesis of some heterocyclic aminoimidazoazarenes. *Food Chem.* **1993**, *46*, 273−276.

(26) Kikugawa, K. Involvement of free-radicals in the formation of heterocyclic amines and prevention by antioxidants. *Cancer Lett.* **1999**, *143*, 123−126.

(27) Kikugawa, K.; Kato, T.; Hiramoto, K.; Takada, C.; Tanaka, M.; Maeda, Y.; Ishihara, T. Participation of the pyrazine cation-radical in the formation of mutagens in the reaction of glucose/glycine/creatinine. *Mutat. Res. Genet. Toxicol. E. M.* **1999**, *444*, 133−144.

(28) Knize, M. G.; Andresen, B. D.; Healy, S. K.; Shen, N. H.; Lewis, P. R.; Bjeldanes, L. F.; Hatch, F. T.; Felton, J. S. Effects of temperature, patty thickness and fat content on the production of mutagens in fried ground beef. *Food Chem. Toxicol.* **1985**, *23*, 1035−1040.

(29) Skog, K.; Jägerstad, M.; Reuterswärd, A. L. Inhibitory effect of carbohydrates on the formation of mutagens in fried beef patties. *Food Chem. Toxicol.* **1992**, *30*, 681−688.

(30) Knize, M. G.; Dolbeare, F. A.; Carroll, K. L.; Moore, D. H.; Felton, J. S. Effect of cooking time and temperature on the heterocyclic amine content of fried beef patties. *Food Chem. Toxicol.* **1994**, *32*, 595−603.

(31) Skog, K.; Steineck, G.; Augustsson, K.; Jägerstad, M. Effect of cooking temperature on the formation of heterocyclic amines in fried meat-products and pan residues. *Carcinogenesis* **1995**, *16*, 861−867.

(32) Skog, K.; Augustsson, K.; Steineck, G.; Stenberg, M.; Jägerstad, M. Polar and nonpolar heterocyclic amines in cooked fish and meat-products and their corresponding pan residues. *Food Chem. Toxicol.* **1997**, *35*, 555−565.

(33) Persson, E.; Sjöholm, I.; Skog, K. Effect of high water-holding capacity on the formation of heterocyclic amines in fried beefburgers. *J. Agric. Food Chem.* **2003**, *51*, 4472−4477.
(34) Persson, E.; Sjöholm, I.; Nyman, M.; Skog, K. Addition of various carbohydrates to beef burgers affects the formation of heterocyclic amines during frying. *J. Agric. Food Chem.* **2004**, *52*, 7561−7566.
(35) Skog, K.; Jägerstad, M. Effects of glucose on the formation of PhIP in a model system. *Carcinogenesis* **1991**, *12*, 2297−2300.
(36) Britt, C.; Gomaa, E. A.; Gray, J. I.; Booren, A. M. Influence of cherry tissue on lipid oxidation and heterocyclic aromatic amine formation in ground beef patties. *J. Agric. Food Chem.* **1998**, *46*, 4891−4897.
(37) Weisburger, J. H.; Veliath, E.; Larios, E.; Pittman, B.; Zang, E.; Hara, Y. Tea polyphenols inhibit the formation of mutagens during the cooking of meat. *Mutat. Res. Genet. Toxicol. E. M.* **2002**, *516*, 19−22.
(38) Weisburger, J. H.; Nagao, M.; Wakabayashi, K.; Oguri, A. prevention of heterocyclic amine formation by tea and tea polyphenols. *Cancer Lett.* **1994**, *83*, 143−147.
(39) Persson, E.; Graziani, G.; Ferracane, R.; Fogliano, V.; Skog, K. Influence of antioxidants in virgin olive oil on the formation of heterocyclic amines in fried beefburgers. *Food Chem. Toxicol.* **2003**, *41*, 1587−1597.
(40) Monti, S. M.; Ritieni, A.; Sacchi, R.; Skog, K.; Borgen, E.; Fogliano, V. characterization of phenolic-compounds in virgin olive oil and their effect on the formation of carcinogenic/mutagenic heterocyclic amines in a model system. *J. Agric. Food Chem.* **2001**, *49*, 3969−3975.
(41) Tsai, S. J.; Jenq, S. N.; Lee, H. Naturally-occurring diallyl disulfide inhibits the formation of carcinogenic heterocyclic aromatic amines in boiled pork juice. *Mutagenesis* **1996**, *11*, 235−240.
(42) Shon, M. Y.; Choi, S. D.; Kahng, G. G.; Nam, S. H.; Sung, N. J. Antimutagenic, antioxidant and free radical scavenging activity of ethyl acetate extracts from white, yellow and red onions. *Food Chem. Toxicol.* **2004**, *42*, 659−666.
(43) Vitaglione, P.; Monti, S. M.; Ambrosino, P.; Skog, K.; Fogliano, V. Carotenoids from tomatoes inhibit heterocyclic amine formation. *Eur. Food Res. Technol.* **2002**, *215*, 108−113.
(44) Murkovic, M.; Steinberger, D.; Pfannhauser, W. Antioxidant spices reduce the formation of heterocyclic amines in fried meat. *Z. Lebensm. Unters. Forsch. A* **1998**, *207*, 477−480.
(45) Nerurkar, P. V.; Lemarchand, L.; Cooney, R. V. Effects of marinating with asian marinades or western barbecue sauce on PhIP and MeIQx formation in barbecued beef. *Nutr. Cancer* **1999**, *34*, 147−152.
(46) Shin, H. S.; Rodgers, W. J.; Gomaa, E. A.; Strasburg, G. M.; Gray, J. I. Inhibition of heterocyclic aromatic amine formation in fried ground beef patties by garlic and selected garlic-related sulfur compounds. *J. Food Prot.* **2002**, *65*, 1766−1770.
(47) Milic, B. L.; Milic, N. B. Protective effects of spice plants on mutagenesis. *Photosynth. Res.* **1998**, *12*, S3−S6.
(48) Tikkanen, L. M.; Latva-Kala, K. J.; Heiniö, R. L. Effects of commercial marinades on the mutagenic activity, sensory quality and amount of heterocyclic amines in chicken grilled under different conditions. *Food Chem. Toxicol.* **1996**, *34*, 725−730.
(49) Salmon, C. P.; Knize, M. G.; Felton J. S. Effects of marinating on heterocyclic amine carcinogen formation in grilled chicken. *Food Chem. Toxicol.* **1997**, *35*, 433−441.
(50) Salmon, C. P.; Knize, M. G.; Felton, J. S.; Zhao, B.; Seow, A. Heterocyclic aromatic amines in domestically prepared chicken and fish from Singapore Chinese households. *Food Chem. Toxicol.* **2006**, *44*, 484−489.
(51) Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biol. Med.* **1999**, *26*, 1231−1237.
(52) Singleton, V. L.; Rossi, J. A., Jr. Colorimetry of total phenolics with phosphomolybdic−phosphotungstic acid reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144−158.
(53) Commission Regulation (EEC) 2676/90 of 17 September 1990 determining Community methods for the analysis of wines. *Off. J. Eur. Communities* **1990**.
(54) Arvidsson, P.; Van Boekel, M. A. J. S.; Skog, K.; Solyakov, A.; Jägerstad, M. Kinetics of formation of polar heterocyclic amines in a meat model system. *J. Food Sci.* **1997**, *62*, 911−916.
(55) Toribio, F.; Puignou, L.; Galceran, M. T. Evaluation of different cleanup procedures for the analysis of heterocyclic aromatic amines in a lyophilized meat extract. *J. Chromatogr. A* **1999**, *836*, 223−233.
(56) Barceló-Barrachina, E.; Moyano, E.; Puignou, L.; Galceran, M. T. Evaluation of different liquid chromatography−electrospray mass spectrometry systems for the analysis of heterocyclic amines. *J. Chromatogr. A* **2004**, *1023*, 67−78.
(57) Cabanis, J. C.; Cabanis, M. T.; Cheynier, V.; Teissedre, P. L. Tablas de composición. In *Enología: Fundamentos Cinetíficos y Tecnológicos*, 2nd ed.; Flancy, C., Ed.; A. Madrid Vicente, Ediciones Mundi-Prensa: Madrid, Spain, 2003; pp 218−226.
(58) Turesky, R. J.; Taylor, J.; Schnackenberg, L.; Freeman, J. P.; Holland, R. D. Quantitation of carcinogenic heterocyclic aromatic amines and detection of novel heterocyclic aromatic amines in cooked meats and grill scrappings by HPLC/ESI-MS. *J. Agric. Food Chem.* **2005**, *53*, 3248−3258.
(59) Solyakov, A.; Skog, K. Screening for heterocyclic amines in chicken cooked in various ways. *Food Chem. Toxicol.* **2002**, *40*, 1205−1211.
(60) Pais, P.; Tanga, M. J.; Salmon, C. P.; Knize, M. G. Formation of the mutagen IFP in model systems and detection in restaurant meats. *J. Agric. Food Chem.* **2000**, *48*, 1721−1726.
(61) Apostolides, Z.; Balentine, D. A.; Harbowy, M. E.; Weisburger, J. H. Inhibition of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]-pyridine (PhIP) mutagenicity by black and green tea extracts and polyphenols. *Mutat. Res.* **1996**, *359*, 159−163.
(62) Zöchling, S.; Murkovic, M.; Pfannhauser, W. Effects of industrially produced flavours with pro- and antioxidative properties on the formation of the heterocyclic amine PhIP in a model system. *J. Biochem. Biophys. Methods* **2002**, *53*, 37−44.
(63) Oguri, A.; Suda, M.; Totsuka, Y.; Sugimura, T.; Wakabayashi, K. Inhibitory effects of antioxidants on formation of heterocyclic amines. *Mutat. Res.* **1998**, *35*, 237−245.
(64) Jones, R. C.; Weisburger, J. H. Inhibiting development of mutagens and carcinogens. U.S. Patent 5011697, 1988; pp 1−13.
(65) Johansson, M. A. E.; Jägerstad, M. Influence of pro- and antioxidants on the formation of mutagenic−carcinogenic heterocyclic amines in a model system. *Food Chem.* **1996**, *56*, 69−75.
(66) Johansson, M.; Jägerstad, M. Influence of oxidized deep-frying fat and iron on the formation of food mutagens in a model system. *Food Chem. Toxicol.* **1993**, *31*, 971−979.
(67) Taylor, R. T.; Fultz, E.; Knize, M. G. Mutagen formation in a model beef supernatant fraction. IV. Properties of the system. *Environ. Health Perspect.* **1986**, *67*, 59−74.
(68) Barnes, W. S.; Weisburger, J. H. Formation of mutagens in cooked foods. VI. Modulation of mutagen formation by iron and ethylenediaminetetracetic acid (EDTA) in fried beef. *Cancer Lett.* **1984**, *24*, 221−226.
(69) Yaylayan, V.; Jocelyn Paré, J. R.; Laing, R.; Sporns, P. In *Intramolecular Nucleoplilic Substitution Reactions of Tryptophan and Lysine Amadori Rearrangement Products*; Finot, P. A., Ed.; Birkhäuser: Basel, Switzerland, 1990; pp 115−120.

Received for review June 12, 2006. Revised manuscript received July 28, 2006. Accepted August 1, 2006.

JF0616311