JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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pubs.acs.org/JAFC

Inhibitory Effect of Antioxidant-Rich Marinades on the Formation of Heterocyclic Aromatic Amines in Pan-Fried Beef

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ABSTRACT: The inhibitory effect of antioxidant-rich marinades containing beer and white wine (with/without alcohol) alone or mixed with herbs commonly used as meat flavoring (garlic, ginger, thyme, rosemary, and red chili pepper) on the formation of heterocyclic aromatic amines (HAs) in pan-fried beef was studied. Radical-scavenging activity was evaluated by DPPH assay, before the addition of meat to the marinade (T0) and after 4 h of meat marinating (T4). At T0, wine with herbs possessed the highest scavenging activity (73.5%), followed by wine (72.5%), dealcoholized wine with herbs (53.4%), beer and herbs (41.7%), dealcoholized wine (39.6%), and beer (25.9%). At T4, a decrease in the radical-scavenging activity of all marinades was observed, although with a similar radical-scavenging profile. All of the six marinades under the study reduced the total amount of HAs, keeping meat with good overall sensory quality. Beer marinades were more efficient than white wine marinades, and the addition of herbs provided a superior inhibitory effect, reducing around 90% of HAs. No correlation was observed between radical-scavenging activity of marinades and total or individual HAs formation. Herbs explained around 30% of inhibition of PhIP formation, whereas alcohol increased PhIP formation.

KEYWORDS: *heterocyclic aromatic amines, herbs, beer, white wine, radical-scavenging activity, sensory analyses*

INTRODUCTION

Cooking meat has a clear beneficial impact, as the microbial content decreases, the digestibility increases, and the flavor and texture improve. However, compounds naturally present can react and under household conditions generate carcinogens, such as heterocyclic aromatic amines (HAs) that are considered important food mutagens/carcinogens.[1]

Several epidemiological studies have shown strong association between the intake of HAs and the risk of important types of human cancer in meat-eating populations, such as cancer of the breast, colon, or pancreas.[2] The International Agency for Research on Cancer (IARC) classified several HAs as probable and possible human carcinogens.[3] When basic human diets include meat, it is impossible to avoid exposure to this group of genotoxic compounds.

HAs can be divided in two classes: aminoimidazoazarenes (AIAs) or thermic HAs with a common structure 2-amino-imidazole moiety and aminocarbolines (ACs) or pyrolitic HAs with 2-aminopyridine as a common structure. HAs formation is the result of complex reactions between creatine, free amino acids, and sugars through the Maillard reaction.[4] The amount of HAs formed in meats depends on meat type, muscle quality, namely, pH, water activity, free amino acids and creatine, and cooking conditions including temperature, time, and equipment used.[4−6] Over the years, both HAs formation pathway and their minimization strategies have been investigated.[4,7−10]

In the last years, special attention was given to antioxidant compounds that contribute to the inhibition of HAs formation and/or their mutagenicity in model systems[11,12] and in real foods.[13,14] Sauces, aromatic herbs, and spices, naturally rich in phenolic compounds, present high antioxidant activity toward free radicals[15] and may provide easy-to-use tools for reduction of HAs dietary intake, when meats are pretreated or cooked with these ingredients.[16] For example, the addition of olive oil,[17−19] tomato,[19] garlic,[20,21] rosemary, thyme, sage, and brine,[14] studied individually, were found to reduce the formation of some HAs in meat. Additionally, marinating meat before cooking with red wine,[8,22] beer,[8] or green tea[9] can be an effective strategy for the reduction of levels of HAs in cooked meat. Moreover, meat marinating with several ingredients is a common practice in several countries for improvement of flavor and tenderness of the cooked product. This pretreatment has the advantage that the cooked meat is not overly spiced and does not develop negative sensory characteristics as only the surface is treated.

Controversial findings on the effect of antioxidant capacity of phenolic compounds or food extracts and HAs formation have been described.[11,12,23] Additionally, information about the effect of mixtures containing antioxidant-rich ingredients, in conditions resembling household reality, is still a challenge for researchers. The ability of marinades containing alcoholic beverages and a mixture of aromatic herbs to minimize the formation of HAs is an important issue for further studies in this area. This study aims to understand the contribution of antioxidant-rich marinades containing beer and white wine (with/without alcohol) alone or mixed with herbs commonly used as meat flavoring (garlic, ginger, thyme, rosemary, and red chili pepper) in the HAs inhibition under household cooking conditions. In addition, meat samples must present adequate

Received: February 9, 2012
Accepted: May 29, 2012
Published: May 29, 2012

sensory characteristics; thus, cooked samples were tested for pleasant flavor by a sensory panel.

MATERIALS AND METHODS

Reagents and Standards. HAs standards, all individual, 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), 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-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), 2-amino-3-methyl-9*H*-pyrido-[2,3-*b*]indole (MeAαC), and 2-amino-6-dimethylpyrido[1,2-*a*:3′,2′-*d*]imidazole (Glu-P-1), were purchased from Toronto Research Chemicals (Toronto North York, ON, Canada). Stock standard solutions of 100 μg/mL in methanol were prepared and used for further dilution.

Sodium hydroxide, hydrochloric acid, ammonium acetate, 25% (v/v) ammonia solution, and triethylamine were of analytical grade and were also purchased from Merck (Darmstadt, Germany). Acetonitrile, methanol, and dichloromethane were of high-performance liquid chromatography (HPLC) grade (Merck).

Ethanol and ethyl acetate of analytical grade were from Merck. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich (St. Louis, MO).

Water was purified with a Milli-Q System (Millipore, Bedford, MA). For solutions. a combined pH glass electrode connected to a pH meter (MicropH 2001, Crison, Barcelona, Spain) and a Magna membrane nylon 0.22 μm were used.

Marinated Meat Samples and Cooking Conditions. *Preparation of Marinades.* TSix different marinades were tested: beer (Bm), beer and herbs (BHm), wine (Wm), wine and herbs (WHm), dealcoholized wine (DWm), and dealcoholized wine and herbs (DWHm). Pilsner beer (5.2% alcohol, made from water, malt, unmalted cereals, and hops) and white wine (13.5% alcohol, produced from Moscatel Galego, Viosinho, Arinto, and Fernão Pires varieties located from Douro valley region) were purchased at local supermarkets. Dealcoholized white wine was obtained using the same white wine, dried on a rotary evaporator at 40 °C (Rotavapor RE 111 equipped with a 461 water bath and Vac V-500 vacuum pump, all from Büchi, Switzerland) and reconstituted with still water. The selected herbs and spices were purchased in a local grocery, and preliminary sensory tests were conducted to adjust their amounts in marinades to achieve good balancing flavors. The following amounts were selected: 100 mL of marinade contained 2.8 g of ginger (*Zingiber officinale*), 2.9 g of garlic (*Allium sativum*), 0.4 g of rosemary (*Rosmarinus officiallis*), 0.25 g of thyme (*Thymus vulgaris*), and 0.1 g of red chili pepper (*Capsicum annumm*). The marinades were prepared immediately before use. Four hours of marinating was selected according to results obtained in previous studies.[7] One group of three meat samples remained unmarinated (control meat samples).

Cooking Conditions. The meat samples, from *Longissimus dorsi* muscle of middle-aged bovine carcasses, were obtained from a major butchery in Porto, Portugal. Meat was chilled overnight in a cooling room (5 ± 1 °C). Following the chilling process, all trimmable fat and connective tissue were removed from the muscle. Steaks were cut manually with similar dimensions (1.2−1.5 cm thick) weighing about 100 g each. The relation between the amount of meat and the volume of marinade was 1:1 (g/mL). Meat samples were marinated during 4 h and pan-fried in a Teflon-coated pan 3 min on each side. The heat source was a gas cooker, and the temperature on the surface of the meat was monitored continuously during cooking with a meat thermometer; it was around 180 °C. Meat was weighed before and after cooking to calculate the percent loss of weight with cooking. Average cooking losses of around 40% were observed. The steaks were cut up using a knife, ground with a food blender, and stored at −20 °C until analysis. Each sample was mixed in a kitchen blender (Moulinex, France) to produce a uniform sample. At the end, the homogenized samples were properly identified and frozen at −20 °C until the analysis. Meat samples were codified as follows: Cb (unmarinated, control beef), Bb (beef marinade with beer), BHb (beef marinade with beer and herbs), Wb (beef marinade with wine), WHb (beef marinade wine and herbs), DWb (beef marinade with dealcoholized wine), and DWHb (beef marinade with dealcoholized wine and herbs).

Determination of Marinades Radical-Scavenging in DPPH Reaction. *Extraction of Phenolic Compounds.* Extraction of phenolic compounds of the marinades was performed according to Zhao et al.[24] and Xanthopoulou et al.,[25] with some modifications. Briefly, marinades at the beginning and at the end of 4 h marinating were analyzed, 15 mL of each batch was extracted during 15 min with 15 mL of ethyl acetate and centrifuged at 1500 rpm for 2 min (Eppendorf 5810 R centrifuge, Eppendorf, Hamburg, Germany), and the supernatant was collected. Then, 6 g of NaCl was added, the pH was set to 1 using 0.05 M HCl, and two extractions were performed, each with 15 mL of ethyl acetate. The supernatants were pooled and evaporated at 40 °C and reduced pressure in a rotary evaporator and redissolved in 10 mL of ethanol 70% (v/v).

Radical-Scavenging Activity Using DPPH. Extracts of Bm, BHm, Wm, WHm, DWm, and DWHm collected at different times were diluted in 96-well microplates (1:4; 1:8). A 100 μL amount of 150 μM DPPH was added to the extracts, and absorbances at 517 nm were recorded for 2 h until the reaction reached a plateau (Biotek microplate reader ELX 808, Biotek Corp., United States). For each extract, two readings with DPPH, $A_{extract}$, and one without the radical, A_{blank1}, were performed. Wells with DPPH solution were used as control, $A_{control}$, and ethanol 70% was used as blank, A_{blank2}. The radical-scavenging activity was expressed as a percentage and calculated with the formula:

$$\%\ \text{DPPH scavenging} = 100 - \left(\frac{A_{extract} - A_{blank1}}{A_{control} - A_{blank2}} \times 100 \right)$$

Analysis of HAs. *Extraction and Purification.* Extraction and purification of HAs were performed according to the method used in interlaboratorial exercises.[26] Sample preparation was as follows: a 5 g sample of pan-fried beef was homogenized in 20 mL of 1 M NaOH with sonication (10 min), and the suspension was then shaken for 1 h using a Vortex Mixer VV3 (VWR International, West Chester, PA). The alkaline solution was mixed with 16 g of diatomaceous earth and was used to fill an empty 20 mL Extrelut column (Extrelut, Merck, Darmstadt, Germany). After it was preconditioned with 7 mL of dichloromethane, a PRS SPE column (Bond Elut PRS, 500 mg, 3 mL from Agilent Technologies, United States) was coupled online to the Extrelut column. To extract the analytes from diatomaceous earth, 75 mL of dichloromethane was passed through the tandem. The washing solutions arising from the PRS cartridge, which consisted of 6 mL of 0.01 M HCl, 15 mL of MeOH, 0.1 M HCl (6:4, v/v), and 2 mL of water, were collected for the analysis of the PhIP and "less polar" compounds (AαC, MeαAC, Trp-P-1, and Trp-P-2). After their organic solvent content was lowered by adding 25 mL of water, the acidic washing solutions were neutralized with 500 μL of ammonia solution. The resulting solution was passed through a 500 mg C_{18} cartridge (Bond Elut C_{18}, from Agilent Technologies), previously conditioned with 5 mL of MeOH and 5 mL of water, and "less polar" amines were concentrated. Finally, the C_{18} cartridge was rinsed with 5 mL of water, and the sorbed HAs were eluted using 1.4 mL of methanol/ammonia solution (9:1, v/v). To collect the "most polar" amines (IQ, Glu-P-1, MeIQx, and 4,8-DiMeIQx), a 100 mg C_{18} cartridge (Bond Elut C_{18}, from Agilent Technologies) was conditioned with 5 mL of MeOH and 5 mL of water and was then coupled online with the PRS cartridge. The "most polar" amines were eluted from the cationic exchanger with 20 mL of 0.5 M ammonium acetate at pH 8.5. Finally, the C_{18} cartridge containing the "most polar" analytes was rinsed with 5 mL of water, and the sorbed HAs were eluted using 0.8 mL of methanol/ammonia solution (9:1) (v/v). Both final extracts containing each group of HAs were gently evaporated under a stream of nitrogen, and the analytes were redissolved in 80 μL of methanol.

Chromatographic Conditions. Separation and quantification of HAs were performed by liquid chromatography with diode array and fluorescence detection (HPLC-DAD/FLD).[5] The chromatographic

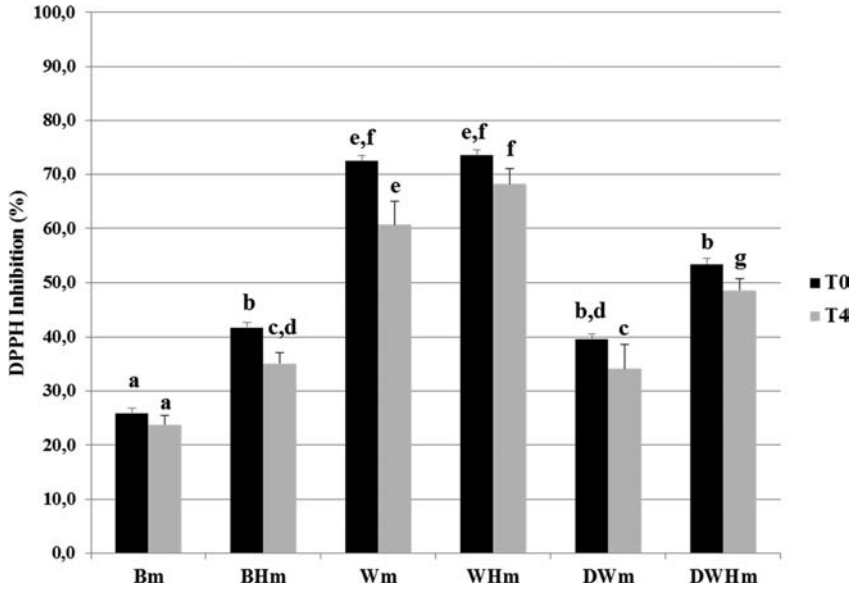

Figure 1. DPPH radical-scavenging activity of marinades under study (Bm, BHm, Wm, WHm, DWm, and DWHm) before the addition of meat to the marinade (T0) and after 4 h of meat marinating (T4). Results are expressed as a percent of inhibition (%) of DPPH. Error bars represent the standard deviation obtained from triplicate experiments. Bars with different letters show significant differences ($p < 0.05$).

analysis was carried out in an analytical HPLC equipped with all unities from Jasco (Japan): one PU-1580 HPLC pump, an autosampler AS-950 with a 20 μL loop, and a MD 910 Multi-wavelength detector (set at 263 nm) coupled to a FP-920 fluorescence detector (excitation, 307 nm; emission, 370 nm). The software used was the Borwin PDA Controller Software (JMBS Developments, Le Fontanil, France).

The separation through the TSK gel ODS80 column (Toyo Soda, Japan) (5 μm; 250 mm length; 4.6 mm internal diameter), at ambient temperature, was performed with the follow mobile phase: solvent A, 0.01 M triethylamine adjusted with phosphoric acid to pH 3.2; solvent B, same as A but adjusted to pH 3.6; solvent C, acetonitrile. The linear gradient program was as follows: 0−10 min, 5−15% C in A, 10−10.1 min exchange of A with B; 10.1−20 min, 15−25% C in B; 20−30 min, 25−55% C in B; 30−55 min, column rinse and re-equilibration.

Peak identification in food samples was carried out by comparing retention times and spectra of unknown peaks with reference standards, as well as cochromatography with added standards and peak purity. Quantification of PhIP, Trp-P-1, MeAαC, and AαC was based on fluorescence peak area. The standard addition method was used for quantification of HAs using the nonspiked sample and two fortified levels (25 and 50 ng of most polar HAs; 50 and 100 ng of less polar HAs and PhIP) before the extraction procedure.

Sensory Analysis. Descriptive analysis was conducted by a trained panel (11 members) to compare the sensory characteristics of meat marinated with beer (Bb), beer + herbs (BHb), wine (Wb), wine + herbs (WHb), and unmarinated control samples (Cb). After they were cooked, the samples were served hot to the sensory panels. Analysis included the evaluation of color, meat odor, beer odor, wine odor, spicy odor, aroma intensity, juiciness, and overall quality. The sensory evaluation was conducted using a 1−7 scale, with 1 representing the lowest intensity and 7 the highest intensity, for all attributes. The sensory panel was composed by master students from University of Porto that had sensory analysis in their curriculum and expressed an interest and disposition to undertake the work. Panelists were trained using marinated and unmarinated beef samples, in four 1 h sessions for term optimization and calibration for accuracy in interpretation and repeatability. Collected data were analyzed by analysis of variance (ANOVA), and panelist deviations were assessed to determine where additional training was needed. In evaluation sessions, samples, including, control and marinated samples, were labeled with random three-digit codes. In each session, panelists received a maximum of five samples to evaluate.

Statistics. The averages of triplicate independent experiments were calculated for each HA. The results were statistically analyzed by ANOVA. Comparison of mean values was made using the Duncan test. Statistical analyses were all performed with SPSS for Windows version 18 (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

Radical-Scavenging Activity of Marinades. The radical-scavenging activity expressed as % of inhibition of DPPH was determined in all marinades under study (Bm, BHm, Wm, WHm, DWm, and DWHm) before the addition of meat to the marinade (T0) and after 4 h of meat marinating (T4), and results are shown in Figure 1. As can be observed, WHm possessed the highest DPPH-scavenging activity before the addition of meat (73.5%), followed by Wm (72.5%), DWHm (53.4%), BHm (41.7%), DWm (39.6%), and Bm (25.9%). The addition of herbs increased the antioxidant activity of marinade medium; however, a significant increase was observed only in beer. After 4 h of meat marinating, a decrease in the radical-scavenging activity of all marinades was observed, although with a similar radical-scavenging profile. However, the decrease was significant only for BHm, DWm, and DWHm. Concerning wine marinades with and without alcohol, the lower radical-scavenging activity of dealcoholized marinades can be attributed to the loss of antioxidant compounds during the reduced pressure evaporation process performed to obtain the dealcoholized wine.[27]

Effect of Marinades in HAs Formation. The total content of HAs in control (unmarinated beef) and 4 h marinated samples (expressed as nmol of HAs/g pan-fried meat) are presented in Figure 2. The total content of HAs is in agreement with literature related with meat samples.[28] All marinades inhibited the amount of total HAs to less than a half of the levels present in control beef. Of the six marinades evaluated, the less effective on reducing the total HAs content was white wine with herbs (WHm). Beer with herbs (BHm) had the strongest inhibitory effect on the total content of HAs. No correlation was found between higher radical-scavenging activity of marinades (either before and after meat marinating)

dx.doi.org/10.1021/jf302227b | J. Agric. Food Chem. 2012, 60, 6235−6240

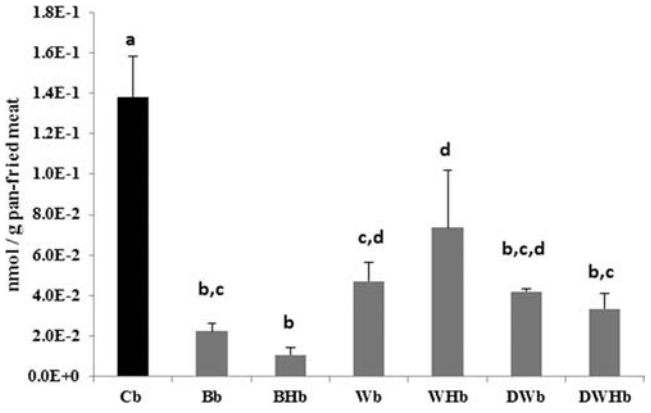

Figure 2. Total HAs formation in control (Cb) and marinated beef samples (Bb, BHb, Wb, WHb, DWb, and DWHb). Results are expressed as nmol HAs/g of pan-fried meat. Error bars represent the standard deviation obtained from triplicate experiments. Different letters above the bars indicate significant differences ($p < 0.05$).

and decrease of total HAs formation. Cheng et al.[12] highlighted that the role of phenolic compounds in Maillard reaction that occurred in HAs formation should be more complex than just being free radical scavenging. Many other factors might contribute to the inhibitory activities of phenolic compounds.

Control beef (unmarinated) exhibit clearly four thermic HAs: IQ, MeIQx, 4,8-DiMeIQx, and PhIP, as shown in Table 1. Pyrolytic HAs, Trp-P-1, Trp-P-2, and Glu-P-1 were identified only in concentrations near the detection limit, and the α-carbolines (AαC and MeAαC) were detected in control beef and all marinated samples at negligible levels (not quantified or around 1 ng/g). Although these HAs were included in total HAs estimation (Figure 2), their contents were not used to evaluate the effect of marinades in these HA individually. Detection and quantification limits were, respectively, 0.06 and 0.56 for Trp-P-1, 0.2 and 2.6 for Trp-P-2, 0.4 and 3.3 for Glu-P-1, and 0.2 and 0.25 for AαC and MeAαC (values expressed as ng/g and determined in our previous work[7]).

PhIP and MeIQx, the most abundant HAs formed under normal cooking conditions,[4] were present in almost equal amounts. PhIP, MeIQx, and 4,8-DiMeIQx results in control samples (Table 1) were similar to pan-fried beef reported by other groups.[12,29−32] IQ was found in control samples in a relatively high amount, and a similar result was observed by Melo et al.[5] and Balogh et al.[33]

The discriminative contribution of individual HAs in each marinade treatment is shown in Table 1; additionally, results are expressed as % of inhibition toward control beef. The detection and quantification limits (expressed in ng/g) were, respectively, 0.02 and 0.25 for PhIP, 0.2 and 1.7 for IQ, 0.06 and 2.6 for MeIQx, and 0.06 and 0.56 for 4,8-DiMeIQx and were already described in our previous work.[7] Only WHb exhibited all four HAs present in control; nevertheless, a significant inhibition toward control was observed. In other marinade treatments, the most carcinogenic HA,[3] IQ, was reduced to levels below the detection limit. 4,8-DiMeIQx was the lowest HA presented in control samples (Table 1); all marinades reduced significantly 4,8-DiMeIQx to values around 1 ng/g or none detected. Concerning these HAs, Ahn and Grün[34] observed a similar behavior in pan-fried beef prior treated with grape seed and rosemary extracts.

PhIP was quantified in all beef samples. In general, marinated beef samples presented significant inhibition of PhIP formation (more than 50%), except Wb and WHb. Beef samples marinated with beer or wine added with herbs (BHb, WHb, and DWHb) presented inhibition of PhIP formation when compared with beef samples marinated in the respective medium alone (Bb, Wb, and DWb). The effect of dealcoholized wine and beer on inhibition of PhIP was statistically similar. Herbs explain around 30% of inhibition of PhIP formation. Murkovic et al.[13] studied the individual application of some of these spices (rosemary, thyme, and garlic) on the surface of meat, kept 24 h prior to cooking that resulted in significantly lower amounts of PhIP. Smith et al.[14] evaluated the effect of three different commercially available marinades and observed a reduction of MeIQx and PhIP due to the spice/herb effect.

MeIQx, the other most abundant HA formed, was reduced in all marinade treatments, with no detection in beer beefs (Bb and BHb); however, herbs did not exhibit an advantage in MeIQx inhibition, especially when added to the wine beefs (Wb and WHb). Murkovic et al.[13] reported a decrease in MeIQx levels because of individual herbs effect.

Concerning white wine, a strong decrease was observed in MeIQx formation, and no effect was observed on PhIP formation. Results obtained for white wine marinade are not in agreement with those obtained previously with red wine,[7,22] and this work describes fairly inhibition on MeIQx and strong inhibition on PhIP formation. According to Busquets et al.,[22] the reducing effect on PhIP formation in red wine marinated may be related to the meat absorption of proline, which is an

Table 1. Effect of Marinades on the Formation of HAs in Pan-Fried Beef at 180 °C after 4 h of Treatment (Results Are Presented as the Mean ± Standard Deviation, $n = 3$) Followed by Inhibition (%)[a]

	HAs (ng/g pan-fried beef) and inhibition (%)			
meat samples	PhIP	IQ	MeIQx	4,8-DiMeIQx
control beef (Cb)	9.69 ± 2.27 a	6.45 ± 0.35 a	9.07 ± 0.6 a	3.60 ± 1.64 a
beer beef (Bb)	4.84 ± 0.93 b,c (50)	ND c (>97)	ND c (>99)	ND b (>98)
beer + herbs beef (BHb)	0.83 ± 0.04 c (91)	ND c (>97)	NQ c (>76)	1.30 ± 0.62 b (64)
wine beef (Wb)	9.82 ± 2.19 a (−1)	ND c (>97)	NQ c (>76)	1.08 ± 1.52 b (70)
wine + herbs beef (WHb)	6.61 ± 2.14 a,b (32)	1.82 ± 0.11 b (72)	6.09 ± 3.27 a,b (33)	1.29 ± 0.48 b (64)
dealcoh. wine beef (DWb)	3.81 ± 1.03 b,c (61)	ND c (>97)	4.38 ± 0.79 b (52)	ND b (>98)
dealcoh. wine + herbs (DWHb)	2.25 ± 1.32 c (77)	ND c (>97)	4.44 ± 0.23 b (51)	ND b (>98)

[a]Means with different letters in the same column are significantly different ($p > 0.05$). Abbreviations ND and NQ stand for not detected and not quantified, respectively. Values in parentheses express % of inhibition towards control beef. When the HAs content of marinated meat was below detection or quantification limits, the percentage of inhibition was estimated using the respective limit of quantification (LOQ) or limit of detection (LOD) as reference values. LOQ and LOD were previously determined.[7].

inhibitor of PhIP formation and was the most abundant amino acid in red wine.

Dealcoholized wine marinades promoted significant reduction of PhIP. Apparently, alcohol seems to perform a strong influence on PhIP formation. According to Busquets et al.,[22] marinating chicken with alcohol/water 1:7 increased the PhIP formation when compared with unmarinated samples. Recently, Wu et al.[35] showed an accelerating capability of ethanol on the formation of IQ and IQx in a dose-dependent manner in model systems and advised that cooking with a high ethanol content may not be safe. No studies were performed for other HAs in model systems.

Sensory Analysis. Sensory analysis was performed on Cb, Bb, BHb, Wb, and WHb samples. In general, data within each attribute were symmetric and mesocurtic. ANOVA performed using the sensory attribute scores was indicative of significant differences in some of the attributes considered. The aforementioned ANOVA indicated that no significant differences were observed for color, juiciness, and overall quality; however, significant differences were noted for all other attributes (meat odor, beer odor, wine odor, spicy odor, and aroma intensity). The mean results obtained by the trained panel for the eight sensory attributes assessed in Cb, Bb, BHb, Wb, and WHb samples are presented in Figure 3. Cb samples

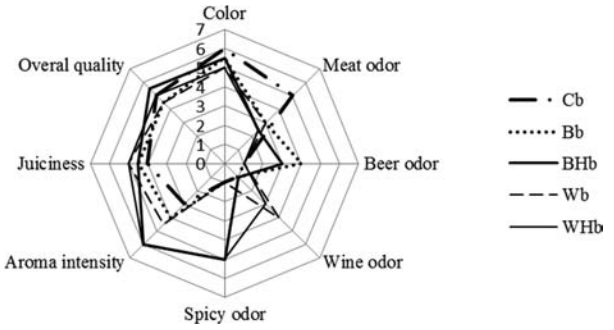

Figure 3. Mean results obtained by a trained panel for the eight sensory attributes assessed in control beef (Cb), beer beef (Bb), beer + herbs beef (BHb), wine beef (Wb), and wine + herbs beef (WHb).

presented higher meat odor ($p < 0.05$). Bb and BHb presented a significantly different beer odor ($p < 0.05$), whereas Wb and WHb presented a significantly different wine odor ($p < 0.05$), and BHb and WHb presented a significantly different spicy odor ($p < 0.05$). However, no significant differences were noted for scores of overall quality ($p > 0.05$), although BHb samples presented the highest score for this attribute.

In conclusion, our data clearly show that all selected marinades exhibited a reduction in total HAs formation in pan-fried meat. In addition, all beef samples presented good overall quality. Beer marinades can be more efficient than white wine marinades, and the addition of herbs provide a superior effect. No correlation was observed between the radical-scavenging activity of marinades and the total or individual HAs formation. In the present study, it was demonstrated that alcohol exerts an important effect on PhIP formation even when applied together with inhibitory ingredients, namely, antioxidant polyphenols.

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Funding

This work has been supported by Fundação para a Ciência e a Tecnologia through PEst-C/EQB/LA0006/2011. O.V. is thankful to FCT, the Portuguese Foundation for Science and Technology, for Ph.D. grant SFRH/BD/42122/2007.

Notes

The authors declare no competing financial interest.

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