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Effect of Beer/Red Wine Marinades on the Formation of Heterocyclic Aromatic Amines in Pan-Fried Beef

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The effect of beer or red wine marinades on the reduction of heterocyclic aromatic amines (HAs) formation in pan-fried beef was compared. The cooking experiments were performed under well-controlled temperature and time conditions. The samples were analyzed for HAs contents using solid-phase extraction and high-performance liquid chromatography−diode array detection/fluorescence detection. Unmarinated samples cooked in similar conditions provided reference HAs levels. Marinating with beer or with red wine resulted in decreased levels of HAs. The amount of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine and 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline reduced significantly, respectively, around 88 and 40% after 6 h of marinating with beer or with wine. High variations were observed for reductions of AαC, ranging between 7 and 77%. Only beer marinade significantly reduced the levels of 4,8-DiMeIQx at 1, 2, and 4 h of marinating. Multivariate statistical treatment of results indicated that beer can be more efficient on the reduction of some HAs formation. In addition, results from descriptive sensory analysis of unmarinated and 2 h marinated beef samples, tested for by two trained sensory panels, pointed to beer marinade as the most adequate for maintaining the usual overall appearance and quality of the pan-fried steaks.

KEYWORDS: Heterocyclic aromatic amines; antioxidants; beer; red wine; meat; marinades; sensory analysis

INTRODUCTION

More than 20 mutagenic/carcinogenic heterocyclic aromatic amines (HAs) have been isolated and identified in cooked foods. These substances are found particular in the crusts of fried, broiled, and cooked meat and fish. The International Agency for Research on Cancer (IARC) has classified several HAs as possible or probable carcinogens and has recommended reducing human exposure to these compounds (*1*).

There are two classes of HAs, aminoimidazole-azaarenes (AIAs) and amino-carbolines (ACs). AIAs formation is the result of complex reactions that involve creatine, free amino acids, and carbohydrates through the Maillard reaction (MR). The development of MR also occurs through a free radical mechanism, which has been shown to play an important role in the formation of AIAs (*2*−*5*). ACs are produced from pyrolysis of proteins or amino acids, such as tryptophan and glutamic acid, heated at high temperature (>250 °C) (*6*). The most popular hypothesis for the formation of ACs under such drastic thermal environment has been a pathway via free radical reactions; however, relatively little investigation has been carried out to verify the above hypothesis as compared with the AIAs (*7*).

The presence of HAs in foods depends on many factors such as type of meat, cooking method, water transport, time, and temperature (*3, 8*). The presence of precursors, enhancers and inhibitors, lipids, antioxidants, and the water content can also influence the formation of HAs (*9*). Meat marinating with several ingredients is used for improvement of flavor, tenderness, and moistness of the cooked product; additionally, they affect the formation of HAs. Earlier works studied the effect of marinating using mixtures of culinary ingredients, and changes in the formation of some HAs were observed (*10, 11*).

Some studies have shown that the concentrations of HAs can also be reduced by addition of compounds with an antioxidant potential. The addition of natural products containing antioxidants that may act as free radical scavengers, such as polyphenols, the main dietary antioxidants, reduces the amount of HAs in the heat-processed meat and model systems. Among these additives, the effect of tea (*12, 13*), red wine (*14*), olive oil (*15*), garlic (*9, 16*), and others has been demonstrated. The hypothesis for their action has been that these inhibitors act against the free radicals generated during HAs formation, preventing the mutagens formation through radical quenchers

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and free radical scavengers activity. However, antioxidants are known to exert both anti- and pro-oxidative effects depending on their concentrations and interactions with other food components during cooking (*7*). Marinating in red wine may reduce the formation of some HAs in chicken meat (*14*), but the influence on red meat, where higher levels of 2-amino-9H-pyrido[2,3-*b*]indole (AαC) may be formed, and the effect of other commonly used beverages rich in antioxidants, such as beer, remain to be clarified. Additionally, practical aspects must be considered, such as the extent to which a particular modulator might affect the appearance or sensory quality of the food.

Evaluation of influence of marinades on cooked meat organoleptic characteristcs is performed by sensory analysis, using in general descriptive analysis (profiling) (*9*). The consistency of results obtained from different sensory panels is an issue that has been frequently addressed, and the need to demonstrate the reliability of sensory panel results has been brought to the forefront of discussions, because even highly trained panels on a product can be subject to the occasional inconsistency. Thus, with the aim of ensuring consistent sensory work, the use of more than one panel is recommended (*17*).

The objective of this study was to compare the effect of beer marinades and red wine marinades in the reduction of AIAs and ACs formation in pan-fried beef. Unmarinated samples cooked in similar conditions provided reference HA levels. In addition, the influence of beer and red wine marinade in meat organoleptic characteristics was evaluated. Thus, the beef samples were tested for descriptive sensory analysis by two trained sensory panels.

MATERIALS AND METHODS

Materials. The compounds studied were 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-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-5H-pyrido[4,3-*b*]indole (Trp-P-1), 3-amino-1-methyl-5H-pyrido[4,3-*b*]indole (Trp-P-2), AαC, 2-amino-3-methyl-9H-pyrido [2,3-*b*]indole (MeAαC), 2-amino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole (Glu-P-1), and 2-aminodipyrido[1,2-*a*:3′,2′-*d*]imidazole (Glu-P-2), purchased from Toronto Research Chemicals (North York Ontario, Canada). Stock standard solutions of 100 μg/mL in methanol were prepared and used for further dilution.

The methanol, acetonitrile, and dichloromethane were of HPLC grade and were provided by Merck (Darmstadt, Germany). The chemicals used for sample treatment [sodium hydroxide, hydrochloric acid, ammonium acetate, and ammonia solution 25% (v/v)] and for mobile phase triethylamine were of analytical grade and were also purchased from Merck. All of the solutions were measured using a combined pH glass electrode connected to a pH meter (MicropH 2001, Crison, Barcelona, Spain) and passed through a nylon membrane, 0.22 μm, from Magna before injection into the HPLC system.

Extrelut reservoirs and Extrelut HM-N diatomaceous earth refill material were obtained from Merck (Darmstadt, Germany). Bond Elut PRS (500 mg) and endcapped Bond Elut C_{18} (100 and 500 mg) cartridges were from Varian (Harbor City, United States). A Supelco Visiprep and a Visidry SPE vacuum manifold (Supelco, Gland, Switzerland) were used for manipulations with solid-phase extraction cartridges and solvent evaporation, respectively. A Vortex Mixer VV3 (VWR international, United States) and ultrassonic cleaner (FungiLab SA, Barcelona, Spain) were used to homogenize cooked meat.

Preparation of Beef Samples and Pan Frying Conditions. The meat samples used in this research were obtained from the *Longissimus dorsi* muscle of middle-aged bovine carcasses. The meat was obtained from a major butchery in Porto, Portugal. The beef sample was chilled for 24 h in a cooling room (5 ± 1 °C). Following the chilling process, all trimmable fat and connective tissue (epimysium) were removed from the *Longissimus dorsi* muscle. Steaks (0.8−1.0 cm thick) were cut manually to pieces of similar dimensions weighing about 90−100 g each. Two different marinades were tested as follows: pilsner beer (5.4% alcohol, made from water, malt, unmalted cereals, and hops) and red wine (13% alcohol, from Douro valley region, produced with Tinta Roriz, Touriga Nacional, and Touriga Franca grape varieties).

Twenty beef samples were used for determination of HAs, divided by the two marinades (eights beef samples for each marinade) and control samples (four beef samples were not marinated). Samples were marinated during 1, 2, 4, and 6 h at 18 °C, using 350 mL of wine or beer; afterward, they were removed from the marinade and were then dried lightly and pan fried. For each condition studied, two beef steaks were marinated and cooked independently. Control beef samples were treated identically to the test samples, except that they were not marinated. Average cooking losses of around 48−50 and 52−55% were observed for unmarinated and marinated samples, respectively. Meat was weighed before and after cooking to calculate the percent loss of weight with cooking.

Beef samples were fried in a Teflon-coated pan 4 min on each side, without adding oil. 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 ranged from 180 to 200 °C. The steaks were cut up using a knife, ground with a food blender, and stored at −20 °C until analysis. Samples were codified as follows: 1W, 2W, 4W, and 6W, respectively, for 1, 2, 4, and 6 h wine marinades, and 1B, 2B, 4B, and 6B, respectively, for 1, 2, 4, and 6 h beer marinades (two steaks each). CS was for control beef samples.

A total of 270 beef samples were used for sensory tests, including 108 for training and 162 for evaluation sessions. Beef samples were marinated in three different plastic containers, one with pilsner beer and another with red wine, so that all of the steaks could be covered completely by the respective marinade at 18 °C. Control steaks were not marinated. Meat was pan-fried at the same conditions used for determination of HAs.

Determination of HAs. Extraction and purification of HAs were performed according to the method developed by Gross (*18*) and modified by Galceran et al. (*19*), since this procedure is the reference method in interlaboratorial exercises (*20*). Sample preparation was as follows. A 5 g sample of 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. The alkaline solution was mixed with Extrelut refill material (16 g) and was used to fill an empty Extrelut column. After being preconditioned with 7 mL of dichloromethane, an Isolute PRS column 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, MeAαC, 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 C18 cartridge (500 mg), previously conditioned with 5 mL of MeOH and 5 mL of water, and less polar HAs were concentrated. Finally, the C18 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). On the other hand, a 100 mg Bond Elut C18 cartridge was conditioned with 5 mL of MeOH and 5 mL of water and was then coupled online with the PRS cartridge. After that, the most polar amines (Glu-P-1, Glu-P-2, IQ, MeIQ, MeIQx, 4,8-DiMeIQx, 7,8-DiMeIQx, and PhIP) were eluted from the cationic exchanger with 20 mL of 0.5 M ammonium acetate at pH 8.5. Finally, the C18 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). The extracts containing either the most or the least polar analytes were gently evaporated under a stream of nitrogen, and the analytes were redissolved in 80 μL of methanol.

A standard addition method was used for quantification of HAs using two fortified levels (around 5−20 ng g^{-1}) and two nonspiked samples.

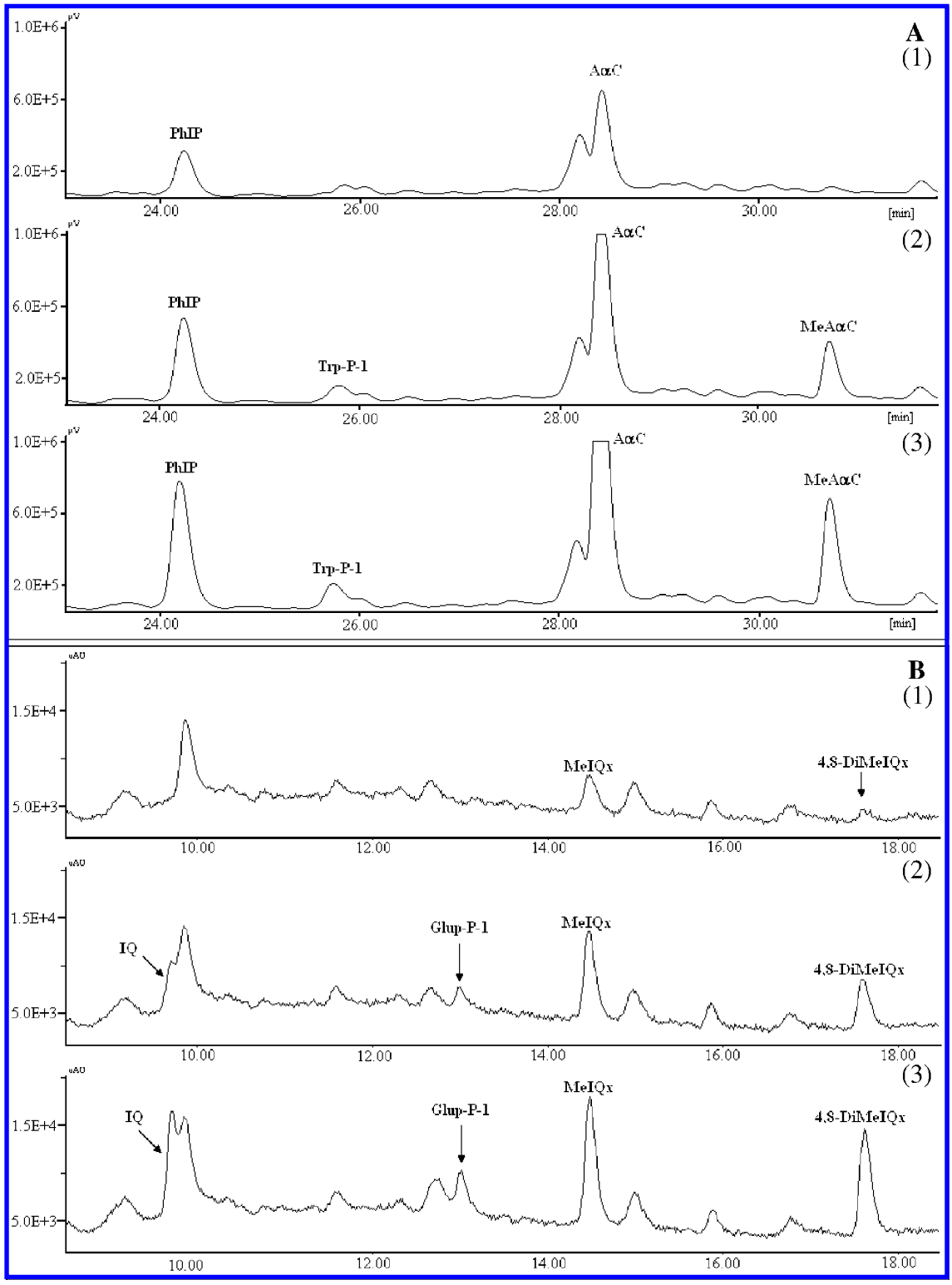

Figure 1. HPLC chromatograms of HAs meat extract. (**A**) Less polar HAs with FLD detector at an excitation of 307 nm and an emission of 370 nm. (**B**) Most polar HAs with DAD at 263 nm. Parts: 1, unspiked; 2, spiked with 5 ng/g for IQ, MeIQx, 4,8-DiMeIQx, and Trp-P-1 and with 10 ng/g for Glu-P-1, PhIP, AαC, and MeAαC; and 3, spiked with 10 ng/g for IQ, MeIQx, 4,8-DiMeIQx, and Trp-P-1 and with 20 ng/g for Glup-P1, PhIP, AαC, and MeAαC.

Separation and quantification of HAs were performed by liquid chromatography with diode array and fluorescence detection (HPLC-DAD/FLD). DAD was set at 263 nm, and the fluorescence detector was set at an excitation of 307 nm and an emission of 370 nm. Quantification of PhIP, MeAαC, and AαC was based on fluorescence peak area. The chromatographic analysis was carried out in an analytical HPLC unit (Jasco, Japan) equipped with one Jasco PU-1580 HPLC pump, a MD 910 Multiwavelength detector, and a type 7125 Rheodyne Injector with a 20 μL loop. The column was a TSK gel ODS80 (Toyo Soda) (5 μm; 250 mm length; 4.6 mm internal diameter). The Borwin PDA Controller Software (JMBS Developments, Le Fontanil, France) was also used. The mobile phase was as follows: 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 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. Separations were carried out at ambient temperature. 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. Triplicate analyses were performed, and the data were subjected to two-way analysis of variance (ANOVA) and principal component analysis using SPSS for Windows, ver. 16 (SPSS, Chicago, IL).

Sensory Tests. Descriptive analysis was conducted by two trained panels (27 members) to evaluate the intensities of the sensory characteristics of the pan-fried samples. After cooking, the samples were served hot to the two sensory panels. Analysis included the evaluation of strange odor, acid, bitter, juiciness, odor intensity, meat odor, red/brown color, overall appearance, wine aroma, beer aroma, adstringency, strange aroma, residual aroma, and overall quality. The sensory evaluation was conducted using a 1−7 scale, with 1 representing the lowest intensity and 7 representing the highest intensity for all attributes. Similar performance was obtained for the two panels (*21*).

The two sensory panels were master students of two different faculties 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. The two panels were trained separately. In session 1, panelists tasted control beef samples with specific highlighted appearance, flavor, and texture attributes. Panelists were invited to generate terms to describe personal observations. In session 2, redundant descriptive terms were removed, and samples exhibiting specific attributes were tasted to include on ballots. Session 3 was designed to establish ballot anchors where all attributes and their synonyms were fitted on an unstructured scale (seven points). To assist panelists, terms were used to describe each attribute at low intensity (score 1) and high intensity (score 7). In session 4, the ballots were tested by panelists in individual booths with unknown representative samples. Collected data were analyzed by ANOVA, and panelist deviations were assessed to determine where additional training was needed. A Learning Management System (WebCT vista, United States) was used for data acquisition during training and evaluation sessions (*21*).

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. The experimental samples were served to panelists in random order, and two evaluation sessions were performed for each panel.

RESULTS AND DISCUSION

HPLC/DAD/FLD for Quantification of HAs. *Quality Control of the Analysis.* The coefficient of variation of the intraday variability for retention times of 14 standards ranged between 0.12 and 1.84%, while the interday variability ranged between 0.33 and 2.75%. For concentration, the coefficient of variation of the intraday variability ranged between 0.33 and 2.75%, while the interday variability ranged between 3.12 and 17.61%. Similar values were obtained by other authors (*22, 23*).

The standard addition calibration curves were within the linearity range of the method. **Figure 1** shows chromatograms of fractions 1 and 2 for spiked and unspiked HAs extracts by DAD and by fluorescence.

The detection limits of 14 HAs standards expressed as ng per injection and based on a signal-to-noise of three were as follows: Glu-P-2 (0.4 ng), IQ (0.2 ng), IQx (0.2 ng), MeIQ (0.2 ng), MeIQx (0.06 ng), 4,8-DiMeIQx (0.06 ng), 7,8 DiMeIQx (0.06 ng), TriMeIQx (0.2 ng), Glu-P-1 (0.4 ng), Trp-P-2 (0.2 ng), PhIP (0.02 ng), Trp-P-1 (0.06 ng), AαC (0.02 ng), and MeAαC (0.02 ng). In food samples, the quantification limits, established as the amount of analyte that produces a signal-to-noise of 10:1, were 5 ng/g for Glu-P-2, 3.3 ng/g for Glu-P-1, 1.7 ng/g for IQ and TriMeIQx, 2.6 ng/g for IQx, MeIQx, and Trp-P-2, 0.86 ng/g for MeIQ, 0.56 ng/g for 4,8-DiMeIQx, 7,8 DiMeIQx, and Trp-P-1, and 0.25 ng/g for PhIP, AαC, and MeAαC.

Concentration of HAs in Control Samples. As a result of previous studies, it was decided to pan fry the meat for 4 min on each side at a measured surface temperature of 180−200 °C, which gave well-done medium brown products with good organoleptic properties (*8*). HAs were analyzed in unmarinated pan-fried meat. PhIP, MeIQx, 4,8-DiMeIQx, and AαC were identified in all samples, at concentrations above the limit of quantification. The concentrations of HAs in pan-fried unmarinated meat samples, expressed on a cooked steak basis, were as follows: PhIP, 33.8 ± 5.5 ng/g; AαC, 19 ± 2.5 ng/g; MeIQx, 3.6 ± 0.5 ng/g; and 4,8-DiMeIQx, 1.3 ± 0.7 ng/g. Trp-P-1, Trp-P-2, and MeAαC were identified but were below quantification limits. The other seven HAs were not detected.

The HAs most frequently found in pan-fried beef are PhIP, MeIQx, 4,8-DiMeIQx, and AαC (*24*). These HAs were quantified in unmarinated samples in levels within the ranges described in selected typical literature data on HAs in common cooked beef. Layton (*25*) reviewed the literature to research concentrations of the principle HAs identified in cooked food; the resultant database contains 261 records categorized by food item, cooking method and conditions, and the HAs detected. Levels of 39, 5.9, and 1.8 ng/g are described, respectively, for PhIP, MeIQx, and DiMeIQx in broiled and fried beefsteak. More recently, Murkovic (*26*) has summarized some of the literature levels of HAs: PhIP levels in red meat are found typically in amounts up to around 35 ng/g, AαC range between 0 and 20 ng/g, MeIQx range between 0 and 10 ng/g, and 4,8-DiMeIQx range between 0 and 5 ng/g. Lower levels of Trp-P-1 and Trp-P-2 are described, between 0 and 1 ng/g. A loss weight between 40−50% is generally referred. Other authors describe similar levels of HAs for beef samples classified as very well done samples (*27*); in this case, the degree of doneness is defined by the internal temperature of samples and not by the weight loss. Sinha (*28*) mentions similar levels for very well done beefsteak with 35% weight loss during cooking at an internal temperature of 93 °C. Other authors refer to lower levels of HAs at similar cooking conditions (*29, 30*) that can be result from different sample dimensions. Several reports have indicated the weight loss may result in increased transport of water-soluble precursors to the surface where the reactions occur (*3*).

It was decided to use as a control unmarinated samples, since this type of sample is usually used by consumers. Additionally, Busquets et al. (*14*) studied the physical effect due to marinating media and analyzed meat samples marinated (30 min, 3 h, and 24 h) prior to cooking in an ethanol/water mixture, with similar alcoholic composition as wine, and no reduction of HAs content was observed as a result of liquid media.

Effect of Marinade Time and Type on the formation of HAs. Pilsner beer and red wine from North of Portugal were used to marinate beef samples to study the effect on HAs reduction. Beer and red wine are rich sources of polyphenols (*31*), from malt and grapes, respectively. Its total content of polyphenols and antioxidant activity has been extensively studied in the past few years. Red wine presents considerably higher polyphenol contents and antioxidant activity when compared with beer (*31*). Marinating with beer or wine can affect the formation of HAs. **Figure 2** displays the concentrations of HAs formed in the unmarinated and different marinated pan-fried meat. Error bars indicate the standard deviation obtained in the quantification of HAs. The same four HAs were quantified in unmarinated

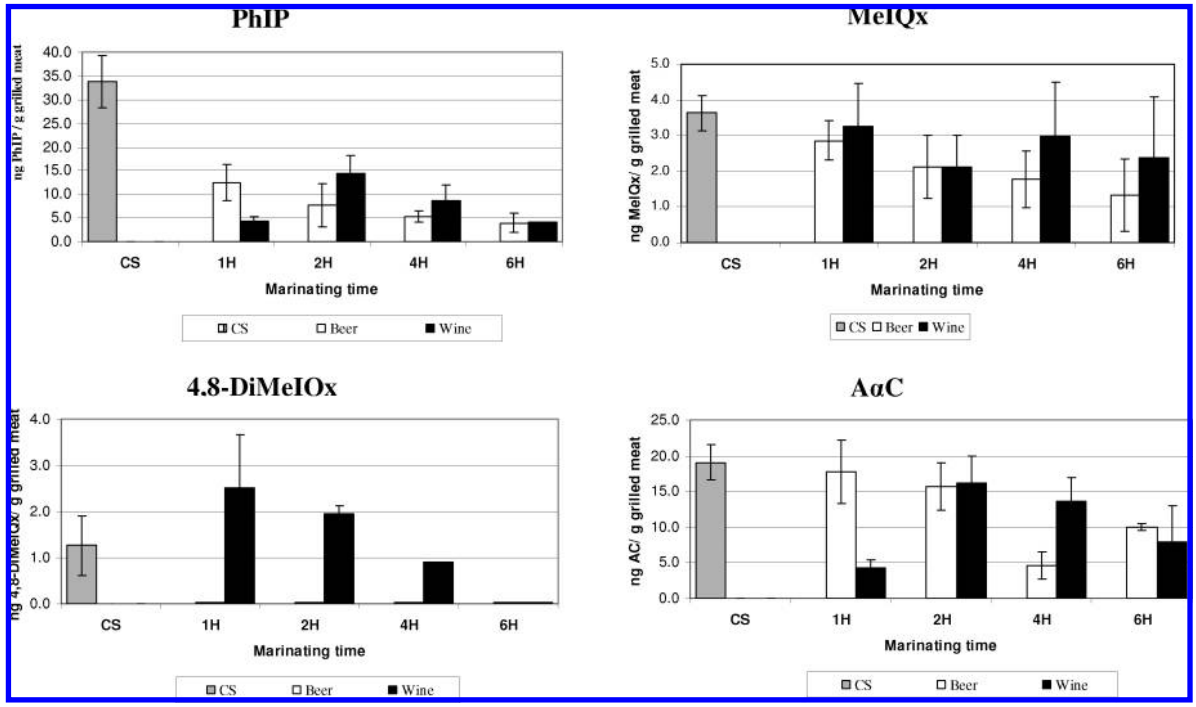

Figure 2. Effect of marinating media and marinating time on HAs formation. Error bars indicate the standard deviation obtained in the quantification of HAs.

and marinated meat. As compared with the unmarinated samples, marinating in beer or in wine resulted in decreased levels of HAs, except for 4,8-DiMeIQx at 1 and 2 h of marinating with wine.

Beer and wine marinades reduced significantly the amount of PhIP ($p < 0.05$), and no significant differences were observed between levels of PhIP of meat marinated with beer and with wine (Tukey test), and the reduction of PhIP levels in meat marinated with the increase of marinating time was not significant ($p = 0.204$) (**Figure 2**).

The reducing effect (88% after 6 h) of wine or beer marinating on the formation of PhIP, the most abundant HAs, was very important. A pronounced reduction of PhIP (83−88%) was also found by Busquets et al. (*14*), using three different types of red wine in fried chicken presenting a high content of PhIP.

In animal studies, an evaluation of the antigenotoxic potential of beer components against carcinogens contained in the human diet, namely, PhIP, was determined (*32, 33*). The results of this study showed that beer components act in a protective capacity against the genotoxic effects of heterocyclic amines in vivo. However, no studies were found concerning the effect of beer marinating on the reduction of PhIP formation.

Beer and wine marinades significantly reduced the amount of MeIQx ($p < 0.05$) after 2, 4, and 6 h of marinating, and higher reduction was observed for beer marinades after 6 h (mean value 44%) when compared with wine marinades (mean value 33%); however, differences between beer and wine reduction were not statistically significant (Tukey test). The reduction of MeIQx levels in meat marinated with the increase of marinating time was not significant ($p = 0.113$).

The reducing effect of wine or beer marinating on the formation of MeIQx was not as remarkable as that observed for PhIP. However, it should be highlighted since the effect of antioxidants in MeIQx formation is still controversial. According to studies for the evaluation of inhibitory effects of antioxidants on the formation of heterocyclic amines, some antioxidants suppressed MeIQx formation, whereas some others promoted MeIQx formation (*34*). An increase of MeIQx formation in pan-fried marinated meat was observed by Busquets et al. (*14*) and by Salmon et al. (*35*) using a different types of marinades. No studies were found concerning the effect of beer marinating on the reduction of MeIQx formation.

The different carbohydrate content of red wine and pilsner beer may also influence the formation of HAs such as MeIQx. The residual sugar content in red wine is generally less than 1.5 g/L, and the polysaccharide level is negligible (*36*), whereas pilsner beer presents around 3.65 g/L of sugars including maltotriose and 24 g/L of dextrines (*37*). It is suggested that maltodextrines can contribute to enhanced water retention due to their ability to imbibe water and, thus, reduce HAs formation (*3*). However, control steaks had one of the lower cooking losses in contrast to marinated samples, and similar results were obtained by Smith (*38*) using commercial marinade packets in grilled steaks.

Concerning 4,8-DiMeIQx, significant differences were observed between levels obtained for meat marinated with beer, wine, and control samples ($p < 0.05$). However, no significant differences were found between control and wine marinade samples (Tukey test). Only beer marinade reduced significantly the levels of 4,8-DiMeIQx at 1, 2, and 4 h of marinating. Wine marinade after 1 and 2 h increased 4,8-DiMeIQx content, but this increase was not significant; after 6 h of wine marinade, the levels of 4,8-DiMeIQx in meat samples were similar to those obtained with beer marinade and near detection limit of the method. The results obtained for wine marinades are in agreement with those found in another study, where inhibition of 4,8-DiMeIQx, up to 87%, was achieved after marinating chicken with wines for long marinating times (*14*).

AαC is present in higher levels in red meat then in other types of meat, such as chicken fillets (*3, 26*), which contain typically between 0 and 1 ng/g; therefore, it was possible to observe the effect of beer and wine marinades on AαC formation, because relatively little investigation has been carried out to verify the hypothesis for the formation of AαC

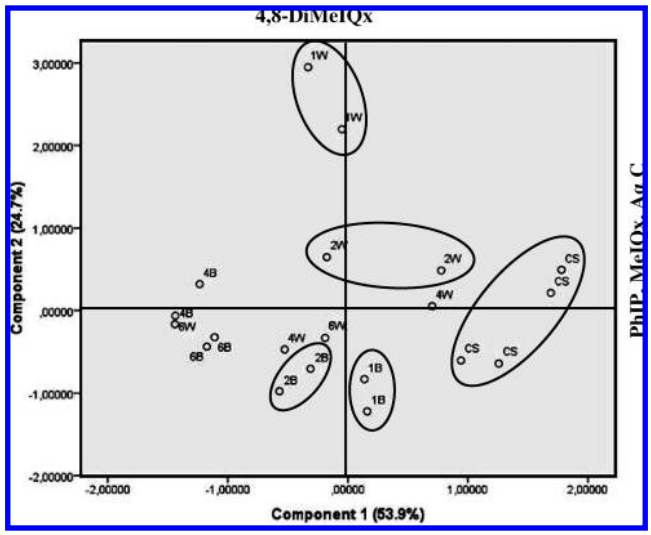

Figure 3. Two-dimensional plot representing the PCA of data from HAs. CS, control samples; 1B, pan-fried steak samples marinated with beer for 1 h; 2B, pan-fried steak samples marinated with beer for 2 h; 4B, pan-fried steak samples marinated with beer for 4 h; 6B, pan-fried steak samples marinated with beer for 6 h; 1W, pan-fried steak samples marinated with wine for 1 h; 2W, pan-fried steak samples marinated with wine for 2 h; 4W, pan-fried steak samples marinated with wine for 4 h; and 6W, pan-fried steak samples marinated with wine for 6 h.

through a pathway via free radical reactions. The two marinades reduced significantly the amount of AαC ($p < 0.05$); no significant differences were observed between levels of AαC of meat marinated with beer and with wine (Tukey test), but significant differences were observed concerning levels of AαC in meat marinated at different time ($p = 0.007$). High variations were observed for reductions of AαC, ranging between 7 and 77%. Person correlation indicates a negative correlation between beer marinating time and concentration of AαC ($p = -0.659$, significant at 0.05 levels); a similar result was not observed for wine marinade. No studies were found concerning the effect of polyphenols on inhibition of AαC formation on meat; however, according to studies of effects of vitamin C, α-tocopherol, and BHT (butylated hydroxytoluene) on the formation of heterocyclic amines in fried fish, inhibition or enhancement of AαC levels depended on the type of antioxidant and concentration (*39*).

The compounds Trp-P-1, Trp-P-2, and MeAαC were identified only in concentrations near the detection limit of the analytical method, so that no statistical evaluation was possible. Principal component analysis (PCA) was performed using HAs levels as variables to reduce the dimensionality of the data and pinpoint the most important effects of marinade time and type on the formation HAs. The results of PCA are depicted on a two-dimensional plot (**Figure 3**)—which is able to explain 78.6% of the total variance. Component 1 explains 53.9% of the variance in the data, and the positive segment of the plot for this component is closely related to the levels of PhIP, MeIQx, and AαC. Component 2 explained 24.7% of the variance in the data; this dimension is positively related to levels of 4,8-DiMeIQx. In this figure, the HAs needed for the definition of these components are shown on the axis edges, indicating the direction in which their levels increase.

Control samples presented high levels of PhIP, MeIQx, and AαC, whereas marinated samples were positioned in different segments of the plot (**Figure 3**). Beer-marinated samples during

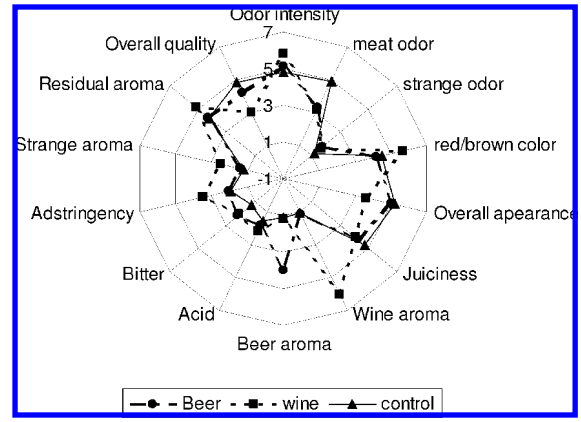

Figure 4. Mean results obtained by the two panels for the 14 sensory attributes assessed in control and beer- and wine-marinated (2 h) pan-fried meat samples cooked according to the description in the Materials and Methods.

1 and 2 h differed from wine-marinated samples, with similar marinating time. For longer marinating times, differences between wine- and beer-marinated samples were lower.

Effect of Beer and Wine Marinades on Sensory Characteristics of Pan-Fried Meat. The increasing requirement for sensory laboratories to show that the results that they provide are repeatable by other laboratories lead to use two different sensory panels. Results obtained by the two panels during training sessions were compared, and after session 4, no significant differences were observed concerning the results. Additionally, the overall conclusions from both analyses were very similar.

Sensory analysis was performed on control pan-fried steaks and on steaks marinated 2 h on wine and beer, because steaks marinated in wine during 4 and 6 h presented very unpleasant wine aromas, strong red color, and poor overall quality, and steaks marinated during 1 h presented higher levels of HAs.

ANOVA performed using the sensory attribute scores was indicative of significant differences ($p < 0.05$) in some of the attributes considered for control samples (unmarinated) and beer- and wine-marinated samples (2 h). In general, data within each attribute were symmetric and mesocurtic. The aforementioned analysis of variance indicated that no significant differences were observed for strange odor, acid, bitter, and juiciness; however, significant differences were noted for all other attributes (odor intensity, meat odor, red/brown color, overall appearance, wine aroma, beer aroma, adstringency, strange aroma, residual aroma, and overall quality). The mean results obtained by the two panels for the 14 sensory attributes assessed in control and beer- and wine-marinated pan-fried meat samples are presented in **Figure 4**.

Control pan-fried steaks presented higher meat odor and higher overall quality when compared with beer- and wine-marinated pan-fried steaks. No significant differences ($p < 0.05$) were observed between beer-marinated pan-fried steaks and control samples concerning red/brown color, overall appearance and quality, adstringency, and strange aroma. Beer-marinated steaks presented a significantly different beer aroma ($p > 0.05$). With respect to wine-marinated pan-fried steaks and control samples, significant differences (Tukey test) were observed for red/brown color, wine aroma, adstringency, and residual aroma. High scores were observed for this attributes. Scores of overall appearance and quality of wine-marinated steaks ($p > 0.05$) were lower when compared with those of beer-marinated steaks and control samples.

In conclusion, our data clearly show that both types of HAs, AIAs, and ACs are affected by beer and red wine marinades.

However, beer marinades can be more efficient on the reduction of some HAs, such as 4,8-DiMeIQx and MeIQx. Additionally, beer marinade has not influenced the usual overall appearance and quality of the pan-fried steaks.

ABBREVIATIONS USED

HAs, heterocyclic aromatic amines; AIAs, aminoimidazole-azaarenes; ACs, amino-carbolines; MR, Maillard reaction; 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; 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; TriMeIQx, 2-amino-3,4,7,8-tetramethylimidazo[4,5-*f*]quinoxaline; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; 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; 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; HPLC-DAD/FLD, high-performance liquid chromatography−diode array and fluorescence detection; IARC, International Agency for Research on Cancer; BHT, butylated hydroxytoluene.

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