Influence of red wine pomace seasoning and high-oxygen atmosphere storage on carcinogens formation in barbecued beef patties

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) and heterocyclic aromatic amines (HAs) are carcinogenic compounds formed in barbecued meat. Conditions that reduce their formation are of major interest. This study aims to evaluate the influence of red wine pomace seasoning (RWPS) and high-oxygen atmosphere storage on the formation of PAHs and HAs in barbecued beef patties. In general, the levels of PAHs and HAs quantified were low. The storage (9 days) promoted higher formation of PAHs in control patties without increase of HAs. RWPS patties cooked at preparation day presented higher levels of PAHs and HAs than control. Nevertheless, RWPS patties cooked after storage presented lower levels of PAHs and HAs than control. ABTS assay pointed out that higher radical scavenging activity may be related to with lower PAHs or HAs formation. In conclusion, RWPS can be an interesting ingredient to inhibit the formation of cooking carcinogens in barbecued patties stored at high-oxygen atmosphere.

KEYWORDS: polycyclic aromatic hydrocarbons, heterocyclic aromatic amines, red wine pomace, beef patties

1. INTRODUCTION

Meat and meat products contain valuable nutrients including proteins, vitamins, iron and zinc. Meat cooking improves the digestibility and prevents microbiological hazards, but also produces carcinogenic chemicals that have drawn the attention of the scientific community. In October 2015, the International Agency for Research on Cancer (IARC) classified "processed meat" and "red meat" as "carcinogenic" and "probably carcinogenic" to humans (groups 1 and 2A), respectively, based on more than 800 epidemiological studies that reported a link between meat consumption and cancer. The potential carcinogenicity of red and processed meat consumption is likely due to a combination of carcinogens present in the meat at the moment of consumption and carcinogens formed in the gastrointestinal tract. Concerning barbecued meat, polycyclic aromatic hydrocarbons (PAHs) and heterocyclic aromatic amines (HAs) were pointed out as two meat components with high carcinogenic potential (Bouvard et al., 2015).

PAHs are mainly formed when meat is cooked over an open flame. The mechanisms are not completely understood, but organic matter seems to be fragmented at high temperatures through pyrolysis. Free radicals are produced and can recombine producing polynuclear aromatic compounds by pyrosynthesis. High temperatures (at least 200ºC) are required to form relevant amounts of PAHs during cooking process (Chen & Chen, 2001; Sharma, Chan, & Hajaligol, 2006). The incomplete combustion of the heat source (charcoal) may generate low molecular weight PAHs, or "light PAHs" (with 2-3 aromatic rings) and the melted fat that drips from patties to the heat source generates PAHs with more than 3 aromatic rings ("heavy PAHs"). These PAHs are carried up by the smoke to the meat surface (Viegas, Novo, Pinto, Pinho, & Ferreira, 2012).

The health risk assessment for PAHs exposure has been addressed by several ways (Yebra-Pimentel, Fernández-González, Martínez-Carballo, & Simal-Gándara, 2015). However, the EU Scientific Committee on Food established the sum of the following PAHs (PAH8): benzo(a)anthracene (BaA), chrysene (CHR), benzo(b)fluoranthene (BbFA), benzo(k)fluoranthene (BkFA), BaP, dibenzo(a,h)anthracene (DBahA), benzo(g,h,i)perylene (BghiP), and indeno(1,2,3-c,d)pyrene (IP) as the most suitable indicator for the carcinogenic potency of PAHs in food (EFSA, 2008).

HAs are another category of carcinogenic compounds formed in cooked meat at high temperature. HAs contain 3 fused aromatic rings, one or more nitrogen atoms in the ring and one exocyclic amino group. HAs are classified as thermic (formed at temperatures between 150 and 250ºC) and pyrolytic (formed at temperatures above 250ºC). The formation of HAs is associated with reaction between creatinine and Strecker degradation products from Maillard reaction (Skog, Johansson, & Jägerstad, 1998).

Packaged raw meat is usually stored under refrigerated conditions in high-oxygen atmosphere to keep the bright red color appreciated by consumers. However, those conditions induce oxidative processes, namely depletion of endogenous antioxidants, lipid and protein oxidation and radical accumulation. Recently, it was described that meat cooked after storage presented higher formation of HAs, which was ascribed to the increase of precursors during storage, especially free amino acids (Polak, Andrenšek, Žlender, & Gašperlin, 2009; Szterk et al., 2012; Szterk & Waszkiewicz-Robak, 2014). Meanwhile, PAHs formation also involves radical reactions, thus the oxidative status of meat may also affect its formation, although the effect of meat storage on PAHs formations during grilling has not been described.

Mitigation strategies to reduce the formation of PAHs and HAs in barbecued meat have been proposed, cooking at lower temperatures, reduction of smoke release and avoid fat dripping (Lee et al., 2016; Skog et al., 1998). Moreover, natural products such as spices and plant extracts that can act as radical scavengers, have been proposed to limit the formation of PAHs and HAs. Recent studies, confirmed that marinating with beer (Viegas, Yebra-Pimentel, Martínez-Carballo, Simal-Gandara, & Ferreira, 2014) or cooking with onion and garlic (Janoszka, 2011) reduced the formation of PAHs, whereas grape seed extract, and wine marinades inhibited HAs formation (Ahn & Grün, 2005; Busquets, Puignou, Galceran, & Skog, 2006; Melo, Viegas, Petisca, Pinho, & Ferreira, 2008; Viegas, Amaro, Ferreira, & Pinho, 2012; Viegas, Moreira, & Ferreira, 2015).

Recently, a new seasoning derived from red wine pomace (RWPS) rich in phenolic compounds, mainly flavonoids (Del Pino-García et al., 2016), presented preservative activity by inhibiting microbial growth, lipid and protein oxidation (Garcia-Lomillo, González-SanJosé, Skibsted, & Jongberg, 2016; García-Lomillo, González-SanJosé, Del Pino-García, Rivero-Pérez, & Muñiz-Rodríguez, 2014). The aim of the present work was to evaluate the effect of RWPS on the formation of PAHs and HAs in barbecued beef patties before and after 9 days of storage in high-oxygen atmosphere.

2. MATERIALS AND METHODS

2.1. Materials

Potassium persulfate ($K_2O_8S_2$) was from Panreac (Barcelona, Spain). 2,2'-Azinobis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS reagent), 6-hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid (Trolox) were obtained from Sigma (St Louis, MO, USA). All the solvents used for PAHs and HAs analysis were of HPLC grade (Merck Darmstadt, Germany) and water was purified using a Milli-Q System (Millipore, Bedford, MA, USA). Hydrochloric acid, ammonium acetate and ammonia solution 25% (v/v) and triethylamine were obtained from Fisher Scientific (Pittsburgh, PA, USA). Extrelut reservoirs and Extrelut HM-N diatomaceous earth refill material were obtained from Merck. The cartridges: Bond Elut PRS (500 mg), Bond Elut C18 (100 and 500 mg) and Mega BE-Si (5 g silica) were obtained from Agilent Technologies (USA). Supelco Visiprep and a Visidry SPE vacuum manifold (Supelco) were used for extraction of PAHs and HAs.

The standard mixture containing naphthalene, acenaphthene, acenaphthylene, fluorene, phenanthrene, anthracene (A), fluoranthene (FA), pyrene (PYR), BaA, CHR, BbFA, BkFA, BaP, DBahA, BghiP, and IP was provided by Supelco (Bellefonte, PA, USA). 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-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole (Glu-P-1) were obtained from Toronto Research Chemicals (North York Ontario, Canada).

2.2. RWPS preparation

RWPS was obtained from dehydrated seedless red wine pomace (González San José, García Lomillo, Del Pino García, Dolores Rivero, & Muñiz Rodríguez, 2015), whose chemical composition as well antimicrobial and antioxidant activities have been previously reported (García-Lomillo et al., 2014). The seasoning was milled (particle size less than 250 µm mesh) and kept in dark until use.

2.3. Patty preparation and cooking

A mixture of different beef cuts, especially sold for the elaboration of patties was obtained from a local supplier (GrosMercat, Burgos, Spain). Doscadesa (Murcia, Spain) provided ingredients and additives used in the formulation (common salt, food grade starch and a commercially available mixture of phosphates).

Patties were made by grounding beef and mixing with additives in a food mixer, according to the following formulations: Control patties (920 g of meat, 12 g of starch, 15 g of salt, 3 g of phosphates, and 50 mL of water) and RWPS patties (same formulation than control patties with the addition of

RWPS to the final concentration of 2% (w/w, seasoning/patty). Finally, patties were manually formed with a thickness between 12 and 15 mm and a weight between 100 and 105 g. Stored samples were placed in polyethylene/ethylene vinyl alcohol/polystyrene trays (Sanviplast, Barcelona, Spain), filled with gas (70% O_2/30% CO_2), and sealed using a polyethylene terephthalate polyvinylidene chloride/polyethylene (PETPVdC/PE) film and kept in dark at 4ºC. Four different groups of patties were formed, Control and RWPS at day 0, which were immediately cooked; and Control and RWPS at day 9, which were stored under refrigeration during 9 days before cooking.

Four different patties were made for each four groups, in order to take into account the intrinsic variability of the patties and of the cooking procedures.

Patties were cooked in a barbecue using wood charcoal, and the temperature was assessed using a thermometer Crison 638 Pt (Barcelona, Spain). When the temperature was 210ºC, samples were placed at 8 cm of distance from the heat source. During patties barbecuing (8 min) the inner temperature of patties was monitored and samples were turned once at 4 min. Charcoal was replaced after cooking each sample.

Raw and cooked patties were weighted and the cooking loss was calculated. The four patties of each group were mixed, homogenized and frozen at -80ºC. Half of the sample was kept frozen for HAs analysis, whereas the other half was freeze-dried for PAHs analysis.

The chemical composition of the raw and cooked beef patties were analyzed using a FoodScan™ near-infrared spectrophotometer (Foss Electric A/S, Hillerød, Denmark) and the data processed by the ISIscan™ Software.

The study was carried out in duplicate on two different days and from two different batches of beef. Thus, two batches per group, each one composed by four patties were barbecued and analyzed three times.

2.4. ABTS$^{\cdot+}$ assay

The radical scavenger activity of raw patties was assessed according to the method described by Rivero-Pérez et al. (2007) adapted to meat samples. The ABTS reagent was prepared by mixing ABTS solution and $K_2O_8S_2$ in Milli Q water (1:1). 75 ± 2 mg of raw patty were mixed with 15 mL of the solution of ABTS reagent and vortexed. After 30 min of reaction with agitation, the radical scavenger activity was evaluated through the absorbance decrease at 734 nm during 30 min. Standard calibration was conducted using Trolox and results were expressed as µmol of Trolox/ g of patty.

2.5. PAHs extraction and quantification

PAHs extraction was carried out according to Viegas, Novo, Pinho, & Ferreira (2012). A HPLC unit (Jasco, Japan) equipped with one PU-1580 HPLC pump, an AS-950 auto sampler with a 20 µL loop and a FP-920 fluorescence detector were used. The system was controlled by Borwin PDA Controller Software (JMBS Developments, Le Fontanil, France). The column was a C18 Supelcosil LC-PAH (25 cm length; 4.6 mm internal diameter, 5 µm particle size) (Supelco, Bellefonte, PA, USA) thermostated at 32ºC. Gradient elution and fluorescence detector excitation/emission program were set up according to Viegas, Novo, Pinho, & Ferreira (2012). Quantification was performed by standard addition method at two fortification levels (10–20 ng/g).

2.6. HAs extraction and quantification

HAs were extracted and purified as described by(Santos et al., 2004). The same HPLC unit (Jasco, Japan) described for PAHs was used, but a diode array detector was also coupled (MD 910 Multiwavelength detector) (HPLC-DAD/FLD). The column was a TSK gel ODS80 (Toyo Soda) (5 µm; 250 mm length; 4.6 mm internal diameter). The mobile phase gradient was set as described by Melo, Viegas, Petisca, Pinho, & Ferreira (2008). DAD was set at 263 nm, whereas FLD was set at excitation 307 nm and emission at 370 nm for quantification of PhIP, MeAαC, and AαC.

Quantification was performed by standard addition method at two fortification levels (5 and 10 ng/g, for thermic HAs and10 and 20 ng/g for pyrolytic HAs).

2.7. Statistical analysis

The effect of seasoning treatment on the concentration of PAHs and HAs and the possible interrelation with the storage treatment was evaluated by a linear model with the fixed effects of seasoning, storage, and the seasoning x storage interaction term. Furthermore, least significant difference (LSD) at a *P*-value < 0.05 was applied to determine statistical differences among the levels of PAHs and HAs of the four groups of patties. Statistical analysis was conducted using IBM SPSS Statistics 19.0 (IBM Corporation, Somers, NY, USA).

3. RESULTS AND DISCUSSION

3.1. Effect of seasoning and storage on total antioxidant capacity of raw patties

Control patties at day 0 presented a radical scavenger activity of 2.98 ± 0.15 µmol/g, whereas after the addition of RWPS it decreased to 1.99 ± 0.11 µmol/g. Del Pino-García et al. (2015) observed that RWPS presented high radical scavenger activity (120 µmol/g of RWPS). However, RWPS blocks thiol groups of proteins that are known to have a predominant role in the antioxidant activity of meat products (Garcia-Lomillo et al., 2016; Serpen, Gökmen, & Fogliano, 2012), which could explain the decrease in the antioxidant activity of RWPS patties. Furthermore, RWPS may induce protein cross-linking of protein tissue and limit the release of endogenous antioxidants from muscle tissue during the assay (Nirmal & Benjakul, 2011).

After 9 days of storage, opposite results were observed. Control samples decreased their radical scavenger activity (1.89 ± 0.17 µmol/g), which may be due to the depletion of endogenous antioxidants such as thiols groups or vitamin E during storage. In contrast, patties with RWPS exhibited higher radical scavenger activity (2.89 ± 0.16 µmol/g) than control samples, and very similar to control at 0 day. This could be explained by the release of phenolic compounds from RWPS matrix during the storage and the protective effect of grape polyphenols on the endogenous chain-braking antioxidants (vitamin E, vitamin C, thiol groups, amine groups or enzymes) (Pazos, González, Gallardo, Torres, & Medina, 2005). Marchiani et al (2016) also observed a gradual release of quercetin during storage of yogurt enriched with grape pomace.

3.2. Patty composition, cooking temperature and cooking loss

Raw patties presented mean values of 64 % of moisture, 18% of protein, 12% of lipid. The incorporation of RWPS did not affect moisture, protein and lipid contents of either raw or cooked patties. Cooked patties presented 57% of moisture, 22% protein and 11% lipid, no significant differences were observed between samples cooked at 0 and 9 days (Table 1). The inner temperature at the end of the cooking was around 83ºC and no significant differences were observed in the temperature profile between control and RWPS samples at 0 and 9 days. The cooking loss averaged 29.8%, without significant differences between the different groups. The results are within the range reported for beef patties (U.S. Department of Agriculture, 2012), but lower than other products such as beef steaks or pork loin that ranged between 40% and 48% (Viegas, Novo, Pinto, et al., 2012). The use of salt, starch, and phosphates increased the water binding and reduced cooking loss (Sebranek, 2009), and this could explain the low levels of cooking loss observed in the present study.

3.3. Effect of RWPS on PAHs formation in barbecued beef patties before and after storage

The PAH8 (EFSA, 2008), as well as FA, PYR and A were quantified in all samples (Table 2). The levels were low, but within the range reported previously for beef steaks (Viegas, Novo, Pinho, et al., 2012; Viegas, Novo, Pinto, et al., 2012). PAHs formation is related to dripping juices from meat to the charcoal and exposition time, the low cooking losses and short cooking time of patties may explain the low level of PAHs found. Additionally, the fat content is an important parameter for

PAHs formation in charcoal grilled muscle foods. Lean beef is known to form lower amount of PAHs compared with pork, chicken and salmon (Viegas, Novo, Pinto, et al., 2012; Viegas et al., 2014). Chung et al. (2011) compared the formation of PAHs in pork and beef, and the PAHs formation in charcoal grilled beef was similar to that obtained in our study, while pork meat produced higher content.

Statistical analysis revealed significant effects and interaction between “seasoning” and “storage” factors. At day 0, RWPS patties presented significantly higher values of A, FA, PYR, CHR, BbFA, BaP and IP than control patties (Table 2). The total amount in RWPS patties (∑PAHs 16.63 ± 0.44 ng/g) was also significantly higher than control samples (∑PAHs 9.67 ± 0.95 ng/g). These results were surprising since antioxidants and spices were reported to mitigate the formation of PAHs (Janoszka, 2011; Viegas et al., 2014). However, RWPS patties at day 0 presented lower scavenging activity against ABTS radical than the corresponding control. Since PAHs formation seems to be mediated by free-radical chain, the lower scavenging activity observed in RWPS patties may explain the higher value of PAHs compared with control. Despite the observed correlation between PAHs formation and ABTS test, it is worth remarking that the mechanisms involved in $ABTS^{\cdot+}$ radical scavenging may be different from those mechanisms taking place during meat cooking. Additionally, the pyrolysis of phenolic compounds may contribute to the higher PAHs measured in RWPS patties. Lignin, cellulose and pectin may render PAHs under pyrolytic conditions (McGrath, Sharma, & Hajaligol, 2001; Sharma & Hajaligol, 2003). RWPS is a complex product with high levels of plant fiber and polyphenols (García-Lomillo et al., 2014) that could explain the higher levels of PAHs measured in RWPS patties at day 0.

Control samples cooked after 9 days of storage presented an increase of PAHs content compared with control patties cooked at day 0 (Table 2). Significant differences were found for FA, PYR and BbFA, and for the sum of quantified PAHs (∑PAHs showed values of 15.78 ng/g and 9.67 ng/g in barbecued patties at 9 and 0 days of storage, respectively). However, RWPS patties cooked after 9 days of storage presented similar PAHs levels (∑PAHs 8.98 ng/g) initially found for control patties. These results could be due to the depletion of endogenous meat antioxidants during storage, which decreased the scavenge capacity against the free radicals of pyrolytic reaction and increased PAHs formation. Furthermore, conjugated dienes, formed during lipid oxidation, may directly undergo dimerization and polymerization leading to the formation of PAHs via Diels-Alder type reactions (Nawar, 1984). No information was found in literature concerning the effect of storage on PAHs formation in barbecued meat, however, Szterk & Waszkiewicz-Robak (2014) demonstrated that raw meat stored for longer periods, formed more HAs during grilling, probably due to the higher content of free amino acids. RWPS patties grilled after 9 days of storage presented lower levels of PAHs than stored control patties, although significant reduction (between 28 and 57 %) was observed only for FA, PYR, BaA, and CHR. RWPS patties cooked at 9 days presented lower levels of A, FA, PYR, BaA, CHR and IP than RWPS patties cooked at 0 days. These results may indicate that the action of polyphenols was not immediate and then some time is required to exert their protective effect. This is in agreement with the ABTS values of RWPS patties that increased from 1.99 µmol/g to 2.89 µmol/g after 9 days of storage. Furthermore, the protection exerted by RWPS to oxidation may contribute to the lower PAHs formation. Previous works have already pointed out the capacity of RWPS to retard the formation of products derived from lipid oxidation, and the loss of endogenous thiols groups (with antioxidant activity) in meat proteins during the storage at high-oxygen atmosphere (Garcia-Lomillo et al., 2016; García-Lomillo et al., 2014).

3.4. Effect of RWPS on HAs formation in barbecued beef patties before and after storage

Among the nine HAs evaluated, only two (PhIP and AαC) were detected above the limit of detection in all samples. Furthermore, MeAαC was also detected in patties cooked with RWPS at day 0 and control samples after 9 days of storage (Table 3). The levels of PhIP and AαC found were within the range reported in beef samples (Szterk et al., 2012). The low formation of HAs in the present study is in agreement with the fact that beef forms lower amounts of HAs than other muscles foods such as chicken or salmon (Viegas, Novo, Pinto, et al., 2012). Additionally, the low cooking loss limited the transfer of precursors to the patty surface where HAs are formed (Skog et al., 1998). The use of starch, salt and phosphate reduced the transport of precursors towards patties surface during cooking (Borgen & Skog, 2004; Persson, Graziani, Ferracane, Fogliano, & Skog, 2003). The short cooking time (4 min each side) and the relatively high thickness of beef patties (10 mm), may also explain the observed low HAs formation. Costa et al. (2009) evaluated the effect of cooking time in HAs formation in charcoal grilled sardines, and observed that no HAs were detected in samples cooked 5 minutes each side even at 280/300 ºC.

Charcoal grilling creates a very dry environment, especially when samples are grilled near to the heat source. PhIP and AαC formation are favored by higher temperatures and dry environment, in opposite these conditions are disadvantageous to the formation of MeIQx and other thermic HAs (Skog, Solyakov, & Jägerstad, 2000). Persson et al (2003) also observed that the addition of NaCl/sodium tripolyphosphate to the beef burgers reduced the cooking loss and decreased the formation of PhIP, MeIQx, and 4,8-DiMeIQx. This decrease was significant for MeIQx and 4,8-DiMeIQx, which may explain that these HAs were not detected in our samples. Starch added to the beef patties inhibited mutagenic activity by up to 54% (Skog, Jägerstad, & Laser Reuterswärd, 1992). The ingredients used may explain the absence of MeIQx in our samples, which is usually found in cooked beef.

At day 0, RWPS patties showed higher contents of PhIP, AαC and MeAαC than control, although the difference was not significant. Control samples cooked after 9 days of storage presented similar values when compared with those from control at day 0 (Table 3). As observed for PAHs, RWPS patties cooked after 9 days of storage presented values of HAs similar to day 0 control patties and lower than those from day 0 RWPS patties. These results agree with the results from ABTS test as described previously. The increased antioxidant activity observed during storage of RWPS patties may also contribute the lower formation of HAs on patties cooked after 9 days of storage, as previously stated for PAHs.

4. CONCLUSIONS

Low levels of PAHs and HAs were found in barbecued beef patties, probably linked to the low cooking loss observed in the samples. Nine days of storage increased PAHs formation in barbecued patties compared with control samples at day 0. RWPS patties cooked after 9 days of storage presented similar PAHs levels found initially for control patties. HAs, other compound with carcinogenic potential, were also evaluated but lower levels of these compounds were quantified and no significant differences were observed in their formation after 9 days of storage. The addition of RWPS during patties storage at high-oxygen may contribute to reduce the formation of carcinogenic compounds after barbecuing in stored samples.

The formation of compounds with potential carcinogenic was reduced in those samples with higher values in the ABTS assay, which may suggest a potential link between the formation of PAHs or HAs and their ability to scavenge different radicals.

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Table

Table 1. Chemical composition and cooking loss of beef patties raw and cooked without red wine pomace seasoning (control) or with red wine pomace seasoning (RWPS).

	Raw patties	Cooked patties			
		Control		RWPS	
		Day 0	Day 9	Day 0	Day 9
Moisture (%)	64.2 ± 0.6	57.0 ± 0.6	57.0 ± 0.2	57.3 ± 0.1	57.9 ± 0.5
Protein (%)	18.3 ± 0.3	22.6 ± 0.6	21.3 ± 0.4	21.7 ± 0.3	20.6 ± 0.6
Lipid (%)	12.4 ± 0.8	9.7 ± 0.3	11.7 ± 0.6	11.0 ± 0.3	10.5 ± 0.4
% Cooking loss		28.6 ± 0.7	29.2 ± 0.7	30.7 ± 1.1	30.7 ± 0.7

[a] Results are presented as the mean ± standard error (n = 2 batches x 3 analytical replicates). No significant differences were observed between cooked samples at a *P*-value < 0.05.

Table 2. PAHs content in barbecued beef patties without seasoning (control) and with seasoning (2% w/w) at day 0 and day 9 storage at high-oxygen atmosphere. [a]

	Control Patties		**RWPS patties**	
	Day 0	**Day 9**	**Day 0**	**Day 9**
Light PAHs				
A	0.86 ± 0.12 a	1.37 ± 0.35 a	2.28 ± 0.19b	0.71 ± 0.05 a
Heavy PAHs				
FA	3.35 ± 0.21 a	7.36 ± 0.85 c	5.42 ± 0.25 b	3.20 ± 0.27 a
PYR	3.04 ± 0.38 a	4.28 ± 0.21 b	5.10 ± 0.14 b	2.40 ± 0.20 a
BaA	0.42 ± 0.06 a,b	0.51 ± 0.04 b,c	0.66 ± 0.05 c	0.32 ± 0.10 a
CHR	0.63 ± 0.05 a,b	0.76 ± 0.09 b	1.30 ± 0.03 c	0.54 ± 0.09 a
BbFA	0.30 ± 0.06 a	0.55 ± 0.06 b	0.53 ± 0.05 b	0.37 ± 0.11 a,b
BkFA	0.19 ± 0.03	0.18 ± 0.01	0.18 ± 0.01	0.18 ± 0.03
BaP	0.22 ± 0.04 a	0.20 ± 0.03 a	0.34 ± 0.05 b	0.23 ± 0.04 a,b
DBahA	0.21 ± 0.03	0.20 ± 0.01	0.18 ± 0.02	0.17 ± 0.03
BghiP	0.30 ± 0.05	0.34 ± 0.08	0.32 ± 0.03	0.24 ± 0.07
IP	0.15 ± 0.02 a	0.24 ± 0.01 a,b	0.33 ± 0.03 b	0.43 ± 0.04 c
∑PAHs	**9.67 ± 0.95 a**	**15.98 ± 0.94 b**	**16.63 ± 0.44 b**	**8.79 ± 0.81 a**

[a] Results are presented as the mean ± standard error (n = 2 batches x 3 analytical replicates). Means with different letters in the same row represent values significantly different (*P*-value < 0.05).

Table 3. HAs content (ng/g) in barbecued beef patties without seasoning (control) and with seasoning (2% w/w) at day 0 and day 9 storage at high-oxygen atmosphere.[a]

	Control Patties		**RWPS Patties**	
	Day 0	**Day 9**	**Day 0**	**Day 9**
PhIP	0.86 ± 0.10 a,b	0.68 ± 0.17 a	1.16 ± 0.21 b	0.69 ± 0.08 a
AαC	0.33 ± 0.04 a,b	0.24 ± 0.01 a	0.49 ± 0.09 b	0.38 ± 0.09 a,b
MeAC	n.q.	n.d.	0.29 ± 0.06	n.d.

[a]Results are presented as the mean ± standard error (n = 2 batches x 3 analytical replicates). Means with different letters in the same row are significantly different (*P*-value < 0.05).n.d.: not detected; n.q: not quantifiable. LOQ (0.02 ng/g) and LOD (0.25 ng/g) were previously determined (Melo et al., 2008)

Highlights

- Red wine pomace avoids the loss in ABTS scavenging activity during meat storage;
- Storage increases the PAHs formation in beef patties;
- Red wine pomace seasoning reduces PAHs formation in stored patties;
- A seasoning that inhibits cooking carcinogens in stored patties is presented.