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# 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.



## 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 >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 200 °C) 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 >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 250 °C) and pyrolytic (formed at temperatures above 250 °C). 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

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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 & Waszkiewicz-Robak, 2014; Szterk et al., 2012). 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 (García-Lomillo, González-SanJosé, Del Pino-García, Rivero-Pérez, & Muñiz-Rodríguez, 2014; Garcia-Lomillo, González-SanJosé, Skibsted, & Jongberg, 2016). 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 <-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 4 °C. 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 210 °C, 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 −80 °C. 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. $ABTS^{\bullet+}$ assay

The radical scavenger activity of raw patties was assessed according to the method described by Rivero-Pérez, Muñiz, and González-Sanjosé (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.