Effect of the inclusion of smoking step in Iberian sausage processing on levels of polycyclic aromatic hydrocarbons

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Abstract— **A traditional Iberian pork sausage, *salchichón, was* elaborated including a treatment of cold smoking for 18 hours using wood from *Quercus ilex*. The levels of six polycyclic aromatic hydrocarbons (PAH) (B(b)F, B(k)F, B(a)A, B(a)P, B(ghi)P and I(123-cd)P) were analyzed at the end of processing. PAH were determined by HPLC-FLD. A control batch that was not smoked was also evaluated. For the control group (n=15) results showed that the six PAH analyzed in this study were not found in detectable amounts. In the smoked batch (n=15) a total sum of the six PAH of 0.4µg/kg were found, being low quantity probably due to the shorter period of smoking than those usually referred in other studies. The 18 hours smoking step incorporated during processing of *salchichón* do not pose any toxicological risk in relation to the presence of PAH.**

Keywords— **Sausage, PAH, smoking.**

I. INTRODUCTION

Polycyclic aromatic hydrocarbons (PAH) are a large group of substances chemically characterized by two or more benzene rings linked together. They are also characterized by high solubility in organic solvents and degraded with light [1]. PAH are formed when organic matter is subjected to a high temperature for long enough time [2]. The concentration of PAH is higher in the smoked products that has been exposured for a longer time to smoke [3], as the wood smoke can contain large amounts of PAH [4]. The importance of this group of compounds is their high carcinogenic potential [5]. About 20 PAH have been shown to have activity as mutagenic, carcinogenic or immunosuppressive [6]. PAH with low molecular weight are considered less dangerous than those with high molecular weight. Fifteen of these compounds (benz (a) anthracene, benzo (b) fluoranthene, benzo (j) fluoranthene, benzo (k) fluoranthene, benzo (g,h,i) perylene, benzo (a) pyrene, chrysene, cyclopenta (c,d) pyrene, dibenz (a,h) anthracene, dibenzo (a,e) pyrene, dibenzo (a,h) pyrene, dibenzo (a,i) pyrene, dibenzo (a,l) pyrene, indeno (1,2,3-cd) pyrene, 5-methylchrysene) were clearly recognized as carcinogenic, for which further investigation of the relative levels was required by a Commission Recommendation [7].

Benzo(a)pyrene (B[a]P) has been identified as very mutagenic and carcinogenic and it has been accepted as a general indicator of total PAH present in wood smoke, smoked foods and environmental samples [8], although it accounts between 1 and 20% of total PAH [9].

Meat products are virtually free of PAH until they are contaminated during processing, especially with smoke or packaging [10].

It is difficult to estimate the real danger of ingestion of smoked products, as PAH levels are highly variable depending on the technology followed and the nature of the product [11, 12]. During the smoking process, the factors that influence the production of PAH are the high temperatures of pyrolysis and the type of wood used [13].

The purpose of this study was to assess the influence of the inclusion of a smoking treatment in the processing of a traditional Iberian pork sausage *salchichón*, on the levels of PAH found in the product.

II. MATERIAL AND METHODS

A. Processing of the sausages and experimental design

Traditional sausages (*salchichón*) were elaborated in an industry of the Iberian sector with Iberian pig fed on acorn and grass prior to slaughter. A total of 30 sausages were made with a standard formulation of chopped pork meat adding other ingredients: salt, corn dextrin, spices (black pepper and nutmeg), dextrose, sodium citrate (E-331), potassium nitrate (E-252) and

sodium nitrite (E-250). The mixture was kept in maceration for 24h at 4ºC and subsequently was stuffed into natural pork casings resulting in sausages of 1.48 ± 0.12kg of weight. Then, the sausages were hung to continue processing. Two different groups were made. Half of the sausages (n=15) followed the traditional process without smoking (control batch). The sausages of the other group (n=15) were treated for cold smoking for 18h (smoked batch). Smoking was carried out in a smoking chamber using *Quercus ilex* wood, controlling and recording temperature and relative humidity (Fig. 1).

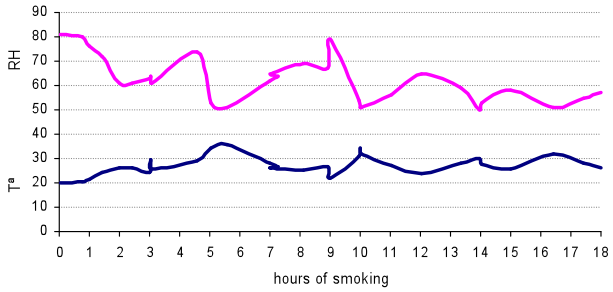

Fig. 1 Temperature (ºC) and relative humidity (%) during the smoking of the sausages

The process followed for both batches the drying stage, which took place in controlled conditions at 10ºC and 75-80% RH for 73 days. The last processing step was made in a cellar, where the sausages stayed under ambient conditions at average temperatures of 15ºC, reaching at times 25ºC to favour the achievement of the sensory characteristics of the product, and RH ranging from 60 to 80%. The process took a total of 135 days.

B. Analysis of polycyclic aromatic hydrocarbons

The levels of PAH after processing were determined by HPLC with fluorescence detection (FLD). Determination was based on the combined methods described by García and Simal [14] with some modifications. For this study six of the 14 PAH clearly recognized as mutagens and carcinogens contained in EC Regulation 1881/2006 were selected: benzo (a) anthracene, benzo (b) fluoranthene, benzo (k) fluoranthene, benzo (a) pyrene, benzo (g,h,i) perylene and indeno (1,2,3-c,d) pyrene, referred by the acronym B(b)F, B(k)F, B(a)A, B(a)P, B(ghi)P and I(123-cd)P, respectively.

Extraction by sonication: To extract PAH from meat, 2.5 g of minced sausage (casing removed) were sonicated with 20ml of hexane for half an hour at ambient temperature. The sample was filtered and sonication was repeated with hexane in the same conditions. After that, the hexane extracts were combined and the resulting 40 ml were submitted to purification process.

Liquid-liquid partition: Hexane extracts were collected in a conical separating funnel where dimethyl sulfoxide (DMSO) was added. 3 times extraction with 10, 5 and 3 ml of DMSO respectively were performed, in order to recover PAH from hexane extract. The hexane layer containing the dissolved fat was discarded. Subsequently, 36 ml of distilled water were added to the pooled DMSO solution and the sample was ready to pass through solid phase extraction cartridges.

Solid phase extraction (SPE) clean-up: Activated C18 bonded phase cartridges (Sep-Pak® Plus C18 cartridge 55-105µm, from Waters) were used for clean-up procedure. The activation was performed passing 5ml of acetonitrile and then 10ml of distilled water through the cartridges. The sample was transferred to the C18 cartridges, which retain PAH, and then for washing, 15ml of acetonitrile/water (1:4) were eluted through the cartridges. Then the cartridges were dried gently with a stream of nitrogen for 15 minutes. Finally, PAH were recollected by elution of 10 ml of hexane through the cartridges. The hexane was removed to dryness by a rotary evaporator under reduced pressure at minimum temperature (35ºC) due to PAH poor thermal stability. The sample was re-dissolved in 500µl of acetonitrile and transferred to a capped amber 2ml vial.

Chromatographic analysis: A 50µl aliquot was injected into an HPLC (Agilent 1100) equipped with autosampler, quaternary pump, thermostated horn and fluorescence detector (G1321A). A C18 reverse phase column for PAH (25cm x 4.6 mm internal diameter x 5µm particle size) from Supelco was used. Constant temperature was maintained at 25 ° C during analysis. The mobile phases were acetonitrile and water at a flow rate of 1ml/min. The gradient conditions are shown in table 1. The wavelengths of excitation and

emission used in fluorescence detection are presented in table 2. The identification of PAH was performed by comparison of retention times with commercial standards analyzed under the same chromatographic conditions as samples. Quantification was carried out by preparing calibration curves for each PAH.

Table 1 Mobile phases gradient used in the HPLC-FLD analysis of PAH

Time (min)	% ACN	% Water
0	60	40
32	90	10
35	97	3
37	100	0
45	60	40
50	60	40

Table 2 Excitation and emission wavelengths program used in the analysis of PAH

PAH	Excitation (nm)	Emission (nm)	Retention time (min)
B(a)A	274	414	24
B(b)F	300	446	31
B(k)F	300	446	34
B(a)P	296	406	37
B(ghi)P	300	470	42
I(123-cd)P	300	470	44

III. RESULTS AND DISCUSSION

The control batch results showed that PAH were not found in detectable amounts (Table 3) considering the six PAH studied in the present study (B(b)F, B(k)F, B(a)A, B(a)P, B(ghi)P and I(123-cd)P). These results are consistent with the fact that these samples did not undergo a period of smoking. On the other hand, in the smoked for 18h batch a total sum of the six PAH of 0.405µg /kg wet weight was found.

Table 3 PAH levels (mean ± sd) in µg/kg of sausage

	Control	Smoked
∑PAH	Not detected	0,405±0,08

These levels are lower than those found in other studies in sausages [14], where, with the same 6 PAH, quantities of 15µg/kg were reached. This difference is probably due to the significantly longer period of sausage smoking that was referred in the latter study, which was 8 days, compared to 18h for our samples of sausages.

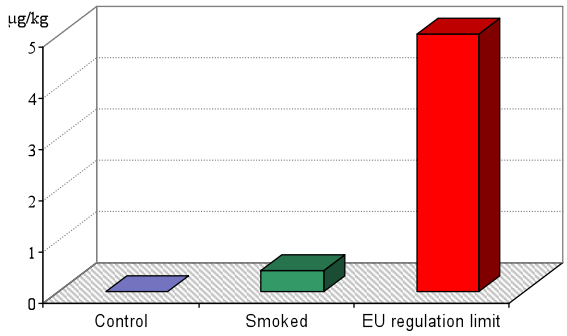

Fig. 2 Sum of PAH level (µg/kg) in sausages of control and smoked batches and B(a)P EU maximum limit

Moreover, our results are consistent with levels described in the review published by Simko [10], where a large quantity of smoked meat products from the market are shown, highlighting a great variability in such values, from 0.03 and reaching even 100µg/kg for B(a)P. In a recent study [15], similar values to those found in our work has been described, being these levels always lower than 0.07µg/kg for each PAH analyzed.

A technical scientific opinion on contaminants in the food chain from EFSA [16] refers that 4 PAH are sufficient to establish the carcinogenic potential. These four PAH are the sum of benzo (a) pyrene, chrysene, benzo (a) anthracene and benzo (b) fluoranthene, being 3 of them analyzed in the present study (benzo (a) pyrene, benzo (a) anthracene and benzo ([b) fluoranthene). The amount achieved of them was well below 5µg/Kg of B(a)P that European Union regulations have established as maximum level for smoked meat and smoked meat product [17].

Given the low amount of PAH found in the samples, it can be said that the consumption of sausages subjected to 18 hours of smoking poses no hazard in relation to the potential effect of tumor induction of PAH. The smoking process has been performed in conditions in which levels of PAH are not remarkable. This fact should be dependent on the type of wood used (oak wood), the mild temperature conditions and the short period of time in which samples have been smoked.

IV. CONCLUSIONS

Smoking for 18h during processing of *salchichón* did not imply a health risk in relation to the presence of PAH, as none of the samples analyzed exceeded the recommended limits by EU legislation.

ACKNOWLEDGMENT

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