Factors affecting levels of PAH in smoked meat products

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Abstract—**In the last years, more studies have been undertaken about the presence of polycyclic aromatic hydrocarbons (PAH) in food, then with factors affecting that contamination. The present work, intended to proceedings which could improve the effect of fat content, casing type and smoking procedures on the PAH prevalence of a dry/fermented cured meat product. Dry fermented sausages were manufactured according to the traditional processing style. Besides fat content variation (10% and 40%), two casing types (tripe and collagen) and two smoking procedures were also tested. The drying stage occurred in an environmental controlled chamber and smoking operation took place during approximately 4 hours/day by direct vs. indirect exposure in a chamber strictly prepared for this purpose. The 16 PAH referred as priority by EPA, were determined by HPLC-UV/Vis-FLD. Total amounts of the 16 PAH ranged between 147 and 870 µg.kg^{-1}, where the light PAH were the most abundant. For the identified carcinogenic PAH, the highest contamination was 10.8 µg.kg^{-1}. However none of the samples revealed contaminations above 5 µg.kg^{-1} (established EU level) for benzo[a]pyrene (BaP). Regardless of the fat content and smoking procedure, samples with collagen casing showed lowest contamination levels. For those made in tripe casing, the lowest PAH content was found in samples with low fat formulation and indirect smoking conditions. The manufacturing practices studied in this work revealed to be effective in minimizing the PAH contamination, contributing to healthier and safer dry smoked meat products.**

Keywords— **PAH, smoked meat products.**

I. INTRODUCTION

Polycyclic aromatic hydrocarbons (PAH) comprise a large group of organic compounds, well known for their carcinogenic and mutagenic properties [1, 2]. These compounds are generated by incomplete combustion of organic matter [3] like diesel, gasoline, coal, or waste incineration, and are often associated with various industrial processes. For this reason they are widespread in the environment: water, air, soils and also in the foods [4]. Besides the environmental pollution [5], foodstuff can also be contaminated during its processing as a result of heat treatments like smoking, drying, roasting, baking or frying [6].

The profile and amount of PAH resulting from the wood burn are influenced by a number of factors such as wood moisture content, oxygen availability, temperature of combustion as well as the type of wood [3, 7]. It is now known that softwoods provide higher levels of PAH [8], namely the heavy ones (with 5 or more fused aromatic rings) that are considered to be the most dangerous.

Deposition and penetration of PAH in smoked foods are also influenced by products water activity [4], fat content [9] and the presence of barriers, such as the casing on the sausages [10].

In last years, benzo[a]pyrene (BaP) has been considered to be a suitable marker for the presence of other PAH in food [8]. Maximum level of BaP in smoked meat products is limited to 5µg.kg^{-1} as defined by EU legislation [11]. More recently, based on the available data, European Food Safety Authority (EFSA) [12] concluded that BaP alone is insufficient as an indicator of PAH occurrence in food and that the sum of 4 or 8 specified (PAH4, PAH8), would be more suitable markers. However, EFSA also concluded that PAH8 (sum of benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene, benzo[g,h,i]perylene, and indeno[1,2,3-cd]pyrene) does not provide much added value when compared to the PAH4 marker (benzo[a]anthracene, chrysene, benzo[b]fluoranthene and benzo[a]pyrene) [12].

With this study we intended to evaluate the influence of fat content, casing type and smoking procedures in PAH contamination levels of traditional smoked meat products.

II. MATERIALS AND METHODS

A. Dry fermented sausages manufacture

Dry fermented sausages were manufactured according to Portuguese traditional processing. Lean and fatty pork trimmings were minced. Mixed with sodium chloride, garlic, paprika, curing salts, antioxidants and water. Two different formulations, with 10% and 40% of fat, and two casing types, collagen and tripe, were tested.

Drying stage alternated between drying chamber (temperature and humidity controlled) and the smoke chamber where the sausages remained for periods of 4h/day until a moisture loss of 30% to 40% was reached. Also two types of smoking regimes were tested: direct and indirect exposure, which consisted in placing/removing an obstacle between the smoke generator and the sausages. The smoke was produced with oak wood (*Quercus ilex L.*), which is widely used in smoking traditional meat products, especially in the South of Portugal.

After processing, sausages were deep frozen (-80 °C) until analysis.

B. PAH analysis

The 16 PAH referred as prioritary by the Environmental Protection Agency (EPA) were analised according the method described by [13]. Briefly samples were thawed (+4 ºC/24h) and homogenized in a Grindomix (GM 200 Retsch, Germany). A sample of 10 g was saponified under reflux with a mixture of potassium hydroxide, methanol and water for 3 hours. Saponified extract was then diluted in 100 mL of a mixture of methanol and water (80:20, v/v) and extracted four times with 50 mL of n-hexane. The extracts containing PAH were combined and evaporated to dryness in a rotary evaporator under reduced pressure. The final residue was dissolved in 3 mL of acetonitrile and filtered through a 0.45 µm membrane (25 mm GHP, Acrodisc, Waters, Milford, MA) and an aliquot injected into chromatographic system for quantification.

Chromatographic separation was carried out in a PAH C18, S-5 µm; 250 x 3.0 mm (Waters, Germany) column coupled to an Alliance Separation Module 2695, using a gradient elution program with ultrapure water obtained from a Milli-Q system (Millipore, Bedford, MA) and acetonitrile HPLC grade from Panreac (Barcelona, Spain). PAH detection was performed in a Multi λ Fluorescence Detector 2475 and a Dual λ UV/Vis Detector 2487 (Waters, Milford, MA).

C. Statistical analysis

The significance of the effects was assessed according General Linear Models using the software Statistica 8.0 (StatSoft Inc., 2007).

III. RESULTS AND DISCUSSION

Mean PAH amounts are showed in Table 1. The sum of the 16 PAH varied in a range of values between 147.23 and 869.66 $\mu g.kg^{-1}$ which were detected in fat formulation and direct smoking samples. Casing type had significant influence over this range, where collagen casing samples showed always significant lower PAH concentrations in regard to tripe ones. For this reason, collagen casing appears to be a better barrier to PAH migration, mainly due to its low fat content [10], as shown in Table 2 where, for total PAH content, casing type revealed to have a most significant effect.

For individual PAH contents, as in previous studies [8, 13], light compounds (ACY, NAP, ACP, FLR, PHE, ANT, FLT, PYR, BaA, CHR) prevailed over the heavy (BbF, BkF, BaP, DhA, BgP and IcP), where the former represented more than 99% of all compounds analysed. Compounds with up to 4 aromatic rings, ACY, NAP, FLR and PHE are the most abundant, while for heavy PAH highest amounts were observed for BbF, BaP and DhA.

BaP in particular had always remained at very low levels (between 0.09 and 0.32 $\mu g.kg^{-1}$), which were below the maximum limit of 5 $\mu g.kg^{-1}$ defined by EU legislation [11].

The PAH4 and PAH8 markers were quite similar. Despite the slightly higher values for the fatty samples, they never exceeded 11 $\mu g.kg^{-1}$. The fat content influence is well depicted in Table 2, where it can be seen as the most significant factor to contribute to PAH4 and PAH8 markers.

Although PAH contents varied among samples the respective profile was very similar between them, probably due to smoke composition, which in turn depends on the conditions by which smoke is generated.

Regarding to smoking procedures, products direct exposure, revealed higher PAH contents, but only for those with tripe casing. Higher permeability of tripe casing favors PAH migration to products inner layers where these compounds could be protected from light decomposition [15].

Statistical analysis also pointed out that first-order interactions Casing type*Formulation and Formulation*Smoking procedures were the ones most significants for Total PAH and PAH4 content, respectively.

Table 1 Mean values (µg.kg^{-1}) for the 16 identified PAH, PAH4 and PAH8 markers, light, heavy and total PAH amounts, for the studied factors.

ACY – acenaphthylene, NAP – naphthalene, ACP – acenaphthene, FLR – fluorene, PHE – phenanthrene, ANT – anthracene, FLT – fluoranthene, PYR – pyrene, BaA – benzo[a]anthracene, CHR – chrysene, BbF – benzo[b]fluoranthene, BkF – benzo[k]fluoranthene, BaP – benzo[a]pyrene, DhA – dibenzo[a,h]anthracene, BgP – benzo[g,h,i]perylene, IcP – indeno[1,2,3-cd]pyrene.

PAH	Lean Formulation				Fat Formulation			
	Tripe Casing		Collagen Casing		Tripe Casing		Collagen Casing	
	Indirect	Direct	Indirect	Direct	Indirect	Direct	Indirect	Direct
ACY	177,45	272,46	130,67	53,08	146,45	342,20	85,44	42,67
NAP	102,45	90,35	22,17	28,80	93,43	246,10	43,11	38,98
ACP	57,26	64,16	15,83	23,48	76,95	117,77	22,36	20,23
FLR	70,89	71,17	10,99	17,55	74,34	99,26	16,37	16,00
PHE	28,86	32,05	8,40	11,82	25,36	29,19	9,11	9,13
ANT	3,32	4,18	0,75	1,16	3,33	5,68	0,82	0,74
FLT	10,51	12,54	4,05	5,69	11,39	7,90	5,38	4,61
PYR	12,01	13,79	4,40	6,23	10,08	10,73	5,75	5,40
BaA	1,99	2,18	3,49	3,30	6,30	7,92	5,73	7,40
CHR	3,88	4,52	0,57	1,10	1,58	1,89	1,01	1,27
BbF	0,20	0,30	0,06	0,17	0,19	0,26	0,19	0,21
BkF	0,05	0,07	0,00	0,04	0,03	0,06	0,04	0,04
BaP	0,32	0,31	0,09	0,21	0,19	0,27	0,23	0,24
DhA	0,35	0,58	0,05	0,21	0,17	0,24	0,39	0,22
BgP	0,06	0,05	0,00	0,05	0,05	0,08	0,04	0,02
IcP	0,11	0,15	0,00	0,05	0,06	0,11	0,07	0,07
PAH4	6,38	7,32	4,21	4,77	8,26	10,35	7,17	9,12
PAH8	6,96	8,17	4,26	5,11	8,58	10,83	7,71	9,47
Light PAH	468,63	567,41	201,33	152,21	449,22	868,64	195,10	146,43
Heavy PAH	0,98	1,32	0,20	0,67	0,63	0,91	0,89	0,73
Total	469,72	568,87	201,53	152,92	449,91	869,66	196,06	147,23

Table 2 Significance of the effects of fat content, casing type and smoking procedures on PAH content.

	PAH4		PAH8		Total	
	F	*p*	F	*p*	F	*p*
Formulation	104,7	0,000	97,6	0,000	4,5	0,044
Casing type	34,8	0,000	42,6	0,000	170,3	0,000
Smoking procedures	21,5	0,000	24,7	0,000	11,0	0,003
Formulation*Casing type	4,1	0,054	8,4	0,008	5,3	0,030
Formulation*Smoking procedures	4,5	0,044	2,5	0,124	6,3	0,019
Casing type*Smoking procedures	0,2	0,673	0,5	0,493	23,5	0,000

IV. CONCLUSIONS

With the present work we concluded that the studied factors may significantly affect PAH contents in smoked meat products, especially casing type and fat content. Since the accumulation of these compounds occurs mainly at the products surface, complementary studies in order to evaluate casing contribution to PAH final concentration in whole product are being developed.

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REFERENCES

1. Wenzl T, Simon R, Kleiner J et al. (2006) Analytical methods for polycyclic aromatic hydrocarbons (PAHs) in food and the environment needed for new food legislation in the European Union. TrAC-Trend Anal Chem 25:716–725
2. Yoon E, Park K, Lee H et al. (2007) Estimation of excess cancer risk on time-weighted lifetime average daily intake of PAHs from food ingestion. Hum Ecol Risk Assess 16:669–680
3. Maga J (1988) Smoke in food processing. CRC Press, Boca Raton, FL
4. Martorell I, Perelló G, Martí-Cid R et al. (2010) Polycyclic aromatic hydrocarbons (PAH) in foods and estimated PAH intake by the population of Catalonia, Spain: Temporal trend. Environ Int 36:424–432
5. Camargo M, Toledo M (2003) Polycyclic aromatic hydrocarbons in Brazilian vegetables and fruits. Food Control 14:49–53
6. Ciecierska M, Obiedzinski M (2007) Influence of smoking process on polycyclic aromatic hydrocarbons content in meat products. Acta Sci Pol, Technol Aliment 6:17–28
7. Roseiro L, Gomes A, Santos C (2011) Influence of processing in the prevalence of polycyclic aromatic hydrocarbons in a Portuguese traditional meat product. Food Chem Toxicol 49:1340–1345
8. Guillén M, Sopelana P, Partearroyo M (2000) Polycyclic aromatic hydrocarbons in liquid smoke flavorings obtained from different types of wood. Effect of storage in polyethylene flasks on their concentrations. J Agric Food Chem 48:5083–5087
9. Moret S, Conte L (2000) Polycyclic aromatic hydrocarbons in edible fats and oils: occurrence and analytical methods. J Chromatogr A 882:245–253
10. García-Falcón M, Simal-Gándara J (2005) Polycyclic aromatic hydrocarbons in smoke from different woods and their transfer during traditional smoking into chorizo sausages with collagen and tripe casings. Food Addit Contam 22:1–8
11. EC (2006) Comission Regulation 1881/2006/EC of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. O J L364:5–24
12. EFSA (2008) Scientific Opinion of the Panel on Contaminants in the Food Chain on a request from the European Commission on Polycyclic Aromatic Hydrocarbons in Food. EFSA J 724:1–114
13. Santos C, Gomes A, Roseiro L (In press) (2011) Polycyclic aromatic hydrocarbons incidence in Portuguese traditional smoked meat products. Food Chem Toxicol
14. Lorenzo J, Purriños L, Fontán M et al. (2010) Polycyclic aromatic hydrocarbons (PAHs) in two Spanish traditional smoked sausage varieties: “Androlla” and “Botillo”. Meat Sci 86:660–664
15. Šimko, P (2002) Determination of polycyclic aromatic hydrocarbons in smoked meat products and smoke flavouring food additives. J Chromatogr B 770:3–18