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Comparative survey of PAHs incidence in Portuguese traditional meat and blood sausages

L.C. Roseiro [a,*], A. Gomes [a], L. Patarata [b], C. Santos [a]

[a] Instituto Nacional dos Recursos Biológicos, I.P. Unidade de Investigação de Tecnologia Alimentar, Estrada do Paço do Lumiar, 1649-038 Lisboa, Portugal
[b] Universidade de Trás-os-Montes e Alto Douro, Quinta dos Prados, Apartado 1013, 5000-911 Vila Real, Portugal

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ABSTRACT

Sixteen polycyclic aromatic hydrocarbons (PAHs) in representative traditional sausages produced in "Trás-os-Montes" and "Alentejo", were determined. Light PAHs represented similar overall contents in both regions and showed close decreasing order patterns (ACY, PHE, FLR and NAP), irrespective of the product type considered. Amongst the carcinogenic/mutagenic PAHs analyzed (PAH8), both regions also had greater contents associated to BaA and CHR, with slightly higher values for the former compound in "Alentejo" and, oppositely, for the later in "Trás-os-Montes". However, their quantitative comparison showed that the general mean total PAH content found in "Trás-os-Montes" was almost 3-fold higher than in similar products from "Alentejo" and this factor was about 8-fold superior when the PAH8 and PAH4 indicators were compared, expressing benzo[*a*]pyrene toxic equivalencies (BaPE), 15 times (total mean toxicity), 34 times (PAH8) and 9 times (PAH4) higher. In general terms, the mean BaP content of all analyzed samples from "Alentejo" was 0.41 μg kg^{-1}. Differently that value in "Trás-os-Montes" reached 3.57 μg kg^{-1}, expressing concerning average contents of 5.35, 5.87 and 4.51 μg kg^{-1} in *Chouriço de Carne*, *Moura* and *Salpicão* sausages, respectively.

1. Introduction

True traditions, meaning those prevailing for generations, are usually supported by valuable reasons. Regarding the smoking operation used for meat and blood products processing at the North of Portugal, clearly more extended and intense than in South region of "Alentejo", the climatic conditions represented, among the influencing factors, probably the most relevant one. Having colder and more rainy winters, the season of excellence for manufacture, people living centuries ago in the mountainous "Trás-os-Montes", certainly had to utilize more frequently the fireplace at home to get a warm environment and concomitantly, to dry out sausages. This was certainly made through the combustion of regional woods, most probably having higher moisture content, affecting the amount of smoke rising up to the products suspended above in the chimney stalk (Tóth and Potthast, 1984; Maga, 1988; Sikorski, 2004). Otherwise, wood species, smoke generation techniques and the ventilation rate evolving at the smoking area would also be different in those regions, affecting the composition of the smoke itself, as well as its deposition rate on products surface (García-Falcón et al., 2004; Garcia-Falcón and Simal-Gándara, 2005; Simko, 2005; Varlet et al., 2007; Viksna et al., 2008; Rey-Salgueiro et al., 2009a). So, distinct specific taste, odor and appearance patterns were being created for the respective products and later on, felt as a tradition. This is what we still think the real present situation is about. Then the risk associated to meat and blood sausages consumption due to the ingestion of PAH contaminants existing in this type of food will, expectably, be distinct.

Many factors have been considered to influence the toxicity effect of a given PAHs contamination degree, mainly the contaminants profile and the occurrence of environmental factors after processing (Simko, 2005). The number of PAHs present in smoked foods can reach near 100 compounds, having effects on living organisms to various extents (Simko, 2005). According to current knowledge, some of them are able to interact in organisms to form PAH derivatives, which are believed to be the ultimate carcinogens, promoters of covalently bound adducts with proteins and nucleic acids, initiating cell mutations and sequent malignancy (Rogan et al., 1993). To simplify an interpretation of the real risk on human health, there have been attempts to express that through the toxic equivalency factors (TEF). Despite the variety and complexity of factors determining the PAHs content variation and evolutionary trends in smoked meat products up to their consumption (Chen and Chen, 2005; Perelló et al., 2009), the study of the incidence of such hazardous compounds seems to be crucial for sequent risk assessment. In this sense, the present work details the PAH contamination "status" of artisanal and more industrial meat/blood sausages manufactured in "Trás-os-Montes", making the

* Corresponding author. Tel.: +351 217127107; fax: +351 217127162.
E-mail address: cristina.roseiro@inrb.pt (L.C. Roseiro).

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comparison with values obtained in products from "Alentejo" (Roseiro et al., 2011; Santos et al., 2011).

2. Materials and methods

2.1. Sampling

A total of 66 samples of smoked meat (*Chouriço de Carne*, *Paínho* and *Paio Tradicional*) and blood (*Chouriço Mouro*, *Cacholeira* and *Morcela*) sausages were obtained from seven producers from "Alentejo", while from the "Trás-os-Montes" region, a total of 42 samples of smoked meat (*Alheira*, *Chouriço de Carne* and *Salpicão*) and blood (*Chouriço Doce*, *Morcela* and *Moura*) sausages were collected from five local producers. All products were filled in natural casings and processed with smoke mostly produced by combustion of *Quercus faginea* and/or *Castanea sativa Miller* wood in "Trás-os-Montes" and of *Quercus ilex* and/or *Quercus suber* wood in "Alentejo". The characteristics of traditional meat and blood products are presented in Table 1.

Direct smoking during the drying operation was generally used in products processing, which were analyzed under the scope of the present study. After smoking/drying operation, products were packed under vacuum to preserve them from further dehydration. Samples were held frozen (−80 °C) and protected from light, until analysis was performed.

2.2. Sample preparation for analysis

For thawing, samples were transferred into a chilling room (4 °C) during approximately 24 h. Thawed products were then hand cut in pieces and finely comminuted in a food cutter/mixer (Grindomix GM200, Retsch, Germany). Samples taken from this homogenized paste were submitted to extraction steps described below.

2.3. Reagents and standards

n-Hexane, methanol and potassium hydroxide used in this study were of analytical grade and acetonitrile was of HPLC grade, all obtained from Panreac (Barcelona, Spain). Water was purified with a Milli-Q System (Millipore, Bedford). PAH standard mixture of 16 PAHs (EPA 610 Polynuclear Aromatic Hydrocarbons Mixture from Supelco (Bellefonte, PA, USA) was used.

2.4. PAHs analysis

The quantification of 16 PAH (naphthalene-NAP; acenaphthylene-ACY, acenaphtene-ACE, fluorene-FLR, phenanthrene-PHE, anthracene-ANT, fluoranthene-FLT, pyrene-PYR, benzo[*a*]anthracene-BaA, chrysene-CHR, benzo[*b*]fluoranthene-BbF, benzo[*k*]fluoranthene-BkF, benzo[*a*]pyrene-BaP, dibenzo[*a*,*h*]anthracene-DhA, benzo[*g*,*h*,*i*]perylene-BgP, indeno[1,2,3-*cd*]pyrene-IcP) was determined by HPLC according to Santos et al. (2011) with modifications. Ten grams of homogenized samples were saponified with a mixture of potassium hydroxide, methanol and water for 3 h under reflux. After this the PAHs were extracted four times with 50 mL of *n*-hexane. The extracts were combined and evaporated using a rotary vacuum evaporator. After evaporation to dryness, the residue was dissolved in 3 mL of acetonitrile and filtered through an Acrodisc membrane 25 mm GHP, GF 0.45 μm (Waters, Milford, MA).

PAHs were separated using a HPLC system consisting of a Separation Module (Waters 2695, Waters, Milford, MA), a fluorescence detector (Waters 2475 Multi λ Fluorescence, Waters, Milford, MA) and a UV/VIS detector (Waters 2487 Dual λ Absorbance detector, Waters, Milford, MA). Separation was performed on a reverse phase PAH C18 column, S-5 μm; 250 × 3.0 mm (Waters, Germany), using a gradient elution program with a mixture of acetonitrile and water which started at 50% acetonitrile, reaching 100% in 20 min and held 100% during 15 min at a flow rate of 1.5 mL min^{-1}. For the PAHs determination, the following detection parameters were used: UV −254 nm (acenaphthylene); fluorescence detector (Ex/Em) 260/366 nm (naphthalene, acenaphtene, fluorene, phenanthrene), 260/430 nm (anthracene, fluoranthene, pyrene, benzo[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, dibenzo[*a*,*h*]anthracene, benzo[*g*,*h*,*i*]perylene) and 270/500 nm (indeno[1,2,3-*cd*]pyrene).

The PAHs quantification in different samples was based in duplicates and carried out through the external standard method. The sum of chrysene, benzo[*a*]pyrene, benzo[*a*]anthracene and benzo[*b*]fluoranthene (PAH4) and the sum of chrysene, benzo[*a*]pyrene, benzo[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, dibenzo[*a*,*h*]anthracene, benzo[*g*,*h*,*i*]perylene and indeno[1,2,3-*cd*]pyrene (PAH8), adopted as a suitable indicators for the occurrence and toxicity of PAHs in food (EFSA Journal, 2008), were calculated. PAHs contents (μg kg^{-1}) were also expressed as benzo[*a*]pyrene toxic equivalencies, according to the converting factors referred in Law et al. (2002).

PAH recoveries and detection and quantification limits (LODs and LOQs, respectively) were determined as described in Santos et al. (2011).

3. Results and discussion

Total levels and respective BaPE values, expressed by the sum of mean contents of the 16 PAHs found in representative traditional meat and blood sausages produced at "Alentejo" and "Trás-os-Montes regions, are shown in Tables 2 and 3, respectively. Light compounds (up to 4 aromatic rings), which are considered harmless to consumers health, represented similar overall relative contents (near 99%) and showed close quantitative order patterns (by decreasing order ACY, PHE, FLR and NAP) in both production regions, irrespective of the product type considered. Among the carcinogenic/mutagenic PAHs analysed (PAH8), both regions had greater contents associated to BaA and CHR, with slightly higher values for the former compound in products from "Alentejo" and, oppositely, for the later contaminant in samples produced in "Trás-os-Montes". Regarding the other compounds integrating PAH8 group, no regular trend was observed for their quantitative order. The justification for these small differences between regions in PAH patterns has to be associated to the dynamics put in the respective drying/smoking procedures, namely concerning the temperature, humidity, volatility and velocity of the smoke stream, which seem to be determinant factors for the smoke composition (Simko, 2005; Rey-Salgueiro et al., 2004, 2008). The PAHs up-taking rate on products surface and finally the contamination degree observed, was also directly correlated with the casing diameter of products in both regions, even if the surface/mass ratios and the fat content of the formulation was comparatively lower, since it determined the time products have to be held in the smoking house. These were the cases represented by *Paio Tradicional* and *Salpicão* from "Alentejo" and "Trás-os-Montes" respectively, which showed the highest mean total PAH contents. Perelló et al. (2009)

Table 1
Characterization of Portuguese traditional meat/blood products.

Portuguese traditional product	Product type	Diameter (cm)	Smoking time (days)	Fat content (%)
Alentejo				
Chouriço de carne	Meat	2	5	25.1
Painho	Meat	4	15	24.2
Paio tradicional	Meat	10	30	40.0
Cacholeira	Blood	2	6	41.8
Chouriço mouro	Blood	2	8	53.0
Morcela	Blood	2	8	46.4
Trás-os-Montes				
Alheira	Meat	2	Unknown	16.0
Chouriço de carne	Meat	2	Unknown	20.6
Salpicão	Meat	6	Unknown	14.4
Chouriço doce	Blood	2	Unknown	11.7
Morcela	Blood	2	Unknown	39.5
Moura	Blood	2	Unknown	23.9