Food and Chemical Toxicology 58 (2013) 369–374

Contents lists available at SciVerse ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Effect of fat content, casing type and smoking procedures on PAHs contents of Portuguese traditional dry fermented sausages

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ARTICLE INFO

Article history:
Available online 18 May 2013

Keywords:
Polycyclic aromatic hydrocarbons (PAHs)
Dry fermented sausages
Fat content
Casing type
Smoking procedures

ABSTRACT

Portuguese dry fermented sausages are traditionally processed through direct drying/smoking, making them susceptible to polycyclic aromatic hydrocarbons (PAHs) contamination. The purpose of this study was to assess the effect of added fat (20% and 40%), casing type (hog and collagen) and smoking procedures (direct and indirect exposure) on the 16 EPA priority PAHs in dry fermented sausages manufactured according traditional processing. The total PAHs content (sum of 16 PAHs) found in whole product (casing included) varied between 150 and 870 μg kg^{-1}, with more than 99% of this content corresponding to harmless low molecular weight compounds. Concerning benzo(a)pyrene (BaP) and PAH4, the respective maximum contents (0.32 and 10.35 μg kg^{-1}, respectively) did not exceed the imposed limits regulated by the European Union. According to our results, casing type was the most influential factor. For hog samples, fat content and smoking regime alone did not influenced the total PAHs amount. However, significantly higher ($p < 0.05$) contamination levels were detected in hog casing samples combining high fat content and direct smoking procedures. In opposition, irrespective of the fat content and smoking regime, safer products, with significantly lower ($p < 0.001$) contamination levels, were obtained when collagen casing was used.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are organic compounds of two or more fused benzenic rings, formed by incomplete combustion of organic matter, namely fossil fuels or wood (Rey-Salgueiro et al., 2008) and are often associated with industrial pollution. PAHs can be found in complex mixtures widely spread throughout the environment (Ishizaki et al., 2010) and, for this reason, humans are usually exposed to these carcinogenic and mutagenic compounds through inhalation, skin absorption and contaminated water and food consumption (Farhadian et al., 2011; Linares et al., 2010). Apart smokers, dietary intake represents the major source of human exposure to PAHs (Farhadian et al., 2011; Martí-Cid et al., 2008). Foods are likely to be contaminated with PAHs from exposure to both environmental pollution and processing practices such as smoking or intense thermal treatments (e.g. drying, roasting, baking or frying) (Camargo and Toledo, 2003; Chung et al., 2011; Rey-Salgueiro et al., 2008).

On smoked foods PAHs contamination levels depend on products characteristics and factors related to smoking process such as wood type and moisture content, oxygen availability and combustion temperature, which influence the amount and profile of formed PAHs (Guillén et al., 2000). Heavy PAHs have five or more fused aromatic rings and due to their stability and toxicity are much more dangerous than the light counterparts (Plaza-Bolaños et al., 2010; Wenzl et al., 2006). High combustion temperatures (Maga, 1988; McGrath et al., 2003) and also the use of softwoods (Guillén et al., 2000; Stumpe-Vīksna et al., 2008a) have been reported to enhance the formation of heavy PAHs. In order to reduce these unwanted compounds, alternative strategies have been tested such as the use of external smoke generators, where the smoke is filtered before being introduced into the smoking room (Duedahl-Olesen et al., 2006; Simon et al., 2010), or even controlling products location within the smoking room (Roseiro et al., 2011).

Despite PAHs accumulate mainly on products surface, due to their lipophilic nature some diffusion can take place to inner layers (Šimko, 2005), where water activity and fat content have a determinant role (Martorell et al., 2010) in migration rate. On the other hand, the presence of barriers such as the casing of smoked sausages (García-Falcón and Simal-Gándara, 2005) and the skin of bacon (Djinovic et al., 2008) can interfere with PAHs migration into products internal layers.

According to European Food Safety Authority (EFSA, 2008), meat and meat products are one of the food categories

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http://dx.doi.org/10.1016/j.fct.2013.05.015

contributing most for dietary intake of PAHs per day of European Union member states consumers. Although previous works have reported that benzo(a)pyrene (BaP) content remains at very low levels (Roseiro et al., 2011; Santos et al., 2011), smoked meat products are very appreciated within the Portuguese population. Therefore these products may represent an important source of exposure to other dangerous PAHs. The current European regulation sets 5 μg kg^{-1} (wet weight) as the maximum level of BaP in smoked meats and smoked meat products (EC, 2006). However, recent data based on the PAHs risk characterization, proved that BaP alone could not be considered as a satisfactory indicator for PAHs occurrence in foods (EFSA, 2008). As a result, from September 2012 a new regulation is applicable for these products (EC, 2011) combining BaP, benzo(a)anthracene (BaA), chrysene (CHR) and benzo(b)fluoranthene (BbFA) contents, known as the PAH4 marker, which was set at 30 μg kg^{-1} (EC, 2011).

The purpose of this research was to evaluate alternatives to the traditional Portuguese manufacturing process of dry fermented sausages, regarding casing type and fat content. On the other hand, considering the metal obstacle that producers often use (in order to avoid melted fat from the hanging products to drop over the smoke source and thus preventing occurrence of fire/flame), two smoking procedures were also tested.

2. Materials and methods

2.1. Dry fermented sausages manufacture

Dry-cured fermented sausages (Portuguese "*chouriço*" type) were manufactured using equipment in a pilot-plant scale. Lean and fatty pork trimmings (obtained from a local producer) were minced and mixed with salt (1%), garlic paste (3%), paprika paste (3.5%), curing salts [0.25% ($NaNO_3$ 4.9%; KNO_3 5%), antioxidants [0.15% (sugars; E301 – relative composition unknown) and water (2.5%)].

A full factorial experimental design was followed, considering as independent variables the fat content (20% and 40% of fat in raw pork trimmings), casing type (collagen and hog casing) and smoking regimes (direct and indirect exposure).

Drying stage took about 8 days until a mean final moisture loss of 35% was reached and occurred both in a controlled environmental chamber (5–15 °C; 25–55% relative humidity) and in a smoking room (with controlled smoke production) where sausages were hold just for 4 h/day.

The tested smoking regimes, direct and indirect exposures, resulted from placing or not an obstacle, a stainless steel plate, above the smoke generator (as can be seen in Fig. 1). The smoke was generated from oak wood (*Quercus ilex L.*), which is widely used for smoking traditional meat products. To ensure similar exposition to smoke action, sticks where products were hanged changed their location within the smoking room according to the scheme represented in Fig. 1b. After processing, sausages were packed under vacuum and deep frozen stored (−80 °C) until analysis ($n = 4$ for each sample type).

2.2. Standards and reagents

The standard mixture of 16 PAHs (EPA 610 Polynuclear Aromatic Hydrocarbons Mix) used for calibrations was purchased from Supelco (Bellefonte, PA, USA). Potassium hydroxide, methanol and n-hexane (analytical grade) and acetonitrile (HPLC-grade) were acquired from Panreac (Barcelona, Spain). Ultrapure water was obtained from a Millipore Milli-Q water purification system.

2.3. PAHs extraction

Before extraction, samples were thawed (+4 °C/24 h) and the casing removed. In order to evaluate PAHs deposition and migration to inner layers, both casing and sausage meat, were independently extracted and quantified. PAHs quantification was performed in a National Reference Laboratory as described by Santos et al. (2011), and further details concerning validation parameters namely limits of detection (LOD) and quantification (LOQ) and recovery can be found in Table 1. Sausage meat was homogenized in a Grindomix (GM 200 Retsch, Haan, Germany). A sample of 10 g was saponified under reflux in presence of a potassium hydroxide, water and methanol mixture. Saponified extract was diluted in 100 mL of a mixture of methanol and water (80:20, v/v) and extracted with 50 mL of *n*-hexane 4 times. The resulting fractions containing PAHs were combined and evaporated to dryness in a rotary evaporator (Laborota 4001, Heidolph, Schwabach, Germany) under reduced pressure. The final residue was dissolved in 3 mL of acetonitrile, filtered through a 0.45 μm membrane (25 mm GHP, Acrodisc, Waters, Milford, MA) and an aliquot (20 μL) injected into chromatographic system for quantification.

The sum of the final PAHs contents in the casing and in the inner layer, taking into account their proportions on meat sausage (casing represents about 1–2% of whole dry fermented sausages) was considered as total PAHs content of the whole product.

2.4. HPLC/UV-FLD analysis

Chromatographic separation of the 16 PAHs (acenaphthylene – ACL, naphthalene – NA, acenaphthene – AC, fluorene – FL, phenanthrene – PHE, anthracene – AN, fluoranthene – FA, pyrene – PY, benzo[*a*]anthracene – BaA, chrysene – CHR, benzo[*b*]fluoranthene – BbFA, benzo[*k*]fluoranthene – BkFA, benzo[*a*]pyrene – BaP, dibenzo[*a,h*]anthracene – DBahA, benzo[*ghi*]perylene – BghiP, indeno[1,2,3-*cd*]pyrene – IP) was done according to Santos et al. (2011).

2.5. Statistical analysis

Data was subjected to analysis of variance (Factorial ANOVA) using Statistica™ v.8.0, software from Statsoft (StatSoft Inc., 1984–2007). Statiscally significant differences ($p < 0.05$) between samples were determined according to Tukey Honest Significant Difference (HSD) test.

3. Results and discussion

Table 2 shows *F* values and statistical significance of individual factors and respective interactions, for each detected PAH content and calculated groups. Among tested factors, casing type had the highest impact in most results ($p < 0.001$), expressed by the high *F* values obtained. Within the heavy PAHs group and concerning casing type, IP, BbFA and BaP stood out with the highest *F* values observed (15341.66, 8590.98 and 1151.48, respectively), while for the low molecular weight compounds, PHE and AN were the most influenced by this factor (1510.89 and 1035.53, respectively). Regardless of fat content and smoking process, on average, the use of collagen casing led to a significant reduction ($p < 0.05$) in total PAHs content, of about 3 times, when compared to samples stuffed in hog casing (Fig. 2). In whole products (casing + inner layer) with high fat content and submitted to direct smoking, the mean total PAHs content ranged from about 147.23–869.66 μg kg^{-1} (Fig. 2), where the found wide range is due to the casing effect. Casing type had also a significant influence ($p < 0.001$) in detected heavy and light compounds (exception for BaA) as well as in PAH4 marker. At the end of processing, samples with collagen casing, had the lowest PAHs content (ranging from 147.22 to 201.52 μg kg^{-1}), showing that the use of synthetic casings contributes to reduce PAHs levels. Products with lower fat content evidenced a decline in light PAHs levels from 468.63 and 567.41 μg kg^{-1} (indirect and direct smoking, respectively) found in hog stuffed sausages to 201.33 and 152.21 μg kg^{-1} (Table 3). A similar trend was observed for heavy PAHs when the synthetic casing was used except for samples with 40% fat and indirectly smoking (Table 3) due to the presence of DBahA in higher levels (about 2-fold). Despite heavy PAHs have been analyzed both in the casing and the inner layer (meat and fat mixture), the respective quantification was only possible in the former portion. This fact is due to PAHs deposition first on the products surface along the drying/smoking process, which then migrates to the inner layers in lower concentrations.

Casing represents only 1–2% of the total mass of traditional dry fermented sausage. However, significantly higher ($p < 0.05$) PAHs contents are found in casings when compared to the inner layer, representing between 5% and 21% of total PAHs content in whole product (Fig. 3). The levels of PAHs detected in casings removed from the sausages are directly related with compounds deposition during smoking process but also with their migration rate into the inner layers. According to our results (Fig. 3), analyzed collagen casings always indicated lower contamination than those observed in hog casings. This fact however, did not result in higher PAHs contents on the inner layers, which leads us to believe that PAHs were deposited in smaller amounts in collagen casing regarding to hog casing. Similar results were reported by Djinovic et al.

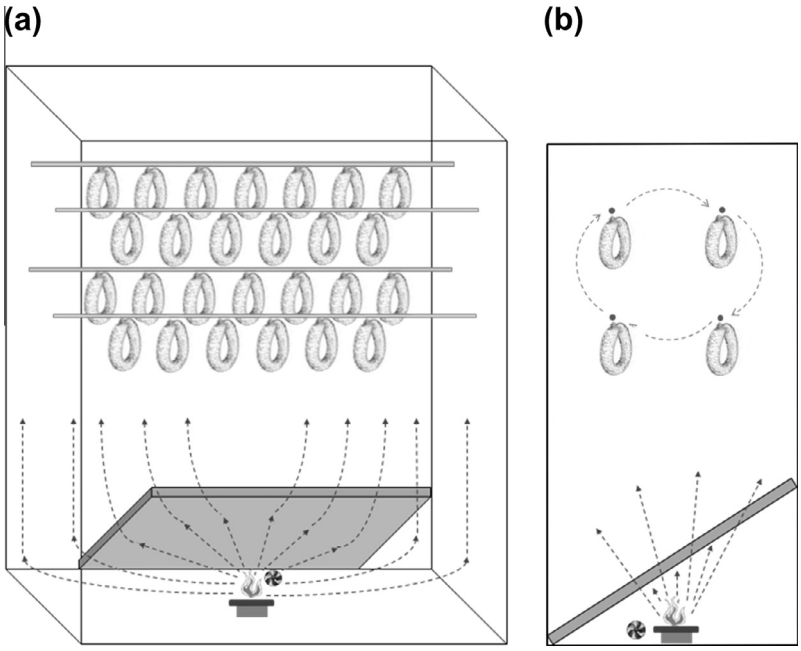

Fig. 1. Graphical representation of the smoking room: (a) front view and (b) side view.

Table 1
LOD (μg kg^{-1}), LOQ (μg kg^{-1}) and recovery (%) of PAHs quantification in meat products.

	LOD	LOQ	Recovery
ACL	18.37	60.61	64.79
NA	0.92	3.03	54.31
AC	0.92	3.03	76.47
FL	0.18	0.61	73.10
PHE	0.09	0.30	82.86
AN	0.09	0.30	97.32
FA	0.18	0.61	98.85
PY	0.09	0.30	96.73
BaA	0.09	0.30	100.20
CHR	0.09	0.30	86.54
BbFA	0.18	0.61	100.41
BkFA	0.09	0.30	100.22
BaP	0.09	0.30	98.86
DBahA	0.18	0.61	88.03
BghiP	0.18	0.61	82.71
IP	0.09	0.30	80.85

(2008) that found different deposition rates for beef and pork ham. On the other hand, as evidenced in Fig. 3, in a percentage basis our results indicate higher contributions of collagen casing to total PAHs level found in whole product (5–21%) when compared to those found in hog casing (6–10%), showing higher permeability in the latter. These findings are in good agreement with the work by García-Falcón and Simal-Gándara (2005) who also found that synthetic casing contributed with higher amounts for total PAHs content of Spanish chorizo and that it prevents more efficiently migration of PAHs from the products surface to products inner layer due to its low fat content.

In addition to lower contamination levels, collagen casing is more easily removed (by comparison to hog casings) reducing consumers' exposure to PAHs. Moreover, while contributing for a higher PAH surface retention, it also allows for PAHs reduction by contacting the packaging material or undergo photodegradation (Šimko, 2005).

Smoking procedures also had a significant impact ($p < 0.05$) in PAHs overall levels (exception found in FA), however it was more markedly expressed in heavy compounds, like BbFA, BkFA, BaP, DBahA and IP ($p < 0.001$) (Table 2). In general, results show that samples directly exposed to the smoke source had higher total PAHs contents when compared with indirectly smoked counterparts, with this increment ranging between 33% and 45% when products stuffed in hog casing were considered. In such condition BbFA, for example, has significantly increased ($p < 0.05$) from 0.20 to 0.30 μg kg^{-1} due to direct smoking process (Table 3). PAHs with 5–6 rings, namely those referred above are less volatile than low molecular weight compounds, and therefore are more susceptible to be retained when an obstacle is placed above the smoke source. Roseiro et al. (2011) has reported the hypothesis that the use of a barrier overhead the smoke source (indirect smoking) could interfere with the smoke ascendant flow and thus reducing PAHs deposition on sausages surface. In fact, the importance of using a physical barrier to avoid the direct exposure of dry fermented sausages to smoke (in order to minimize PAHs contamination, with special emphasis on the most dangerous compounds), is clearly demonstrated by the results of the present study. Yet contributing to the performance of indirect smoking, it must be underlined that to further decrease PAHs formation from pyrolysis of melted fat (from hanging sausages) should be avoided (Chung et al., 2011; Viegas et al., 2012).

Although to a lesser extent, fat content, had also an important effect ($p < 0.05$) in PAHs contamination levels (Table 2). The obtained trends were not always consistent, varying with the casing type and the smoking method. However, its effect was more evident in samples stuffed in hog casing and smoked under a direct regime, resulting in the highest total PAHs levels found (869.66 μg kg^{-1}). Still, for some light PAHs like NA, AC, FL, FA, PY, BaA and CHR the fat content has stood out as the second most important factor. Such effects may be attributed to the lipophilic properties of PAHs (Martorell et al., 2010) since the use of a formulation with higher fat content increases the probability of PAHs migration to the inner layers, particularly when using hog casing.

Factors also interacted significantly for the majority of analyzed PAHs, but the interaction between fat content and casing type ("F × C") emerged as the most significant, namely in what concerns heavy PAHs such as IP, BbFA and BaP. For these compounds, higher

Table 2
F values and statistical significance of the tested factors on PAHs content.

	Fat content (F)	Casing type (C)	Smoking procedures (S)	Interactions			
				F × C	F × S	C × S	F × C × S
ACL	0.05^{ns}	73.39***	5.43*	1.66^{ns}	3.43^{ns}	31.58***	0.81^{ns}
NA	15.19***	76.55***	9.83**	6.42*	11.39**	9.15**	14.80***
AC	94.34***	882.55***	45.59***	78.85***	9.37**	28.66***	30.70***
FL	15.91***	825.90***	12.53**	9.78**	4.00^{ns}	4.60*	12.68**
PHE	17.74***	1510.89***	27.92***	4.88*	1.95^{ns}	3.25^{ns}	4.12^{ns}
AN	7.98**	1035.53***	75.93***	20.97***	6.11*	50.59***	24.22***
FA	11.10**	463.01***	0.32^{ns}	14.61***	56.95***	4.94*	8.75**
PY	14.10***	434.80***	10.68**	21.41***	7.71*	0.64^{ns}	0.78^{ns}
BaA	222.89***	1.94^{ns}	9.00**	11.34**	8.94**	0.09^{ns}	0.15^{ns}
CHR	238.92***	801.68***	38.71***	394.57***	4.64*	0.41^{ns}	0.07^{ns}
BbFA	1405.10***	8590.98***	7374.45***	4060.74***	1321.69***	222.98***	329.34***
BkFA	0.75^{ns}	981.49***	850.93***	749.96***	88.08***	9.66**	247.97***
BaP	0.15^{ns}	1151.48***	419.44***	1131.98***	6.69*	26.56***	363.69***
DBahA	6.68*	60.72***	21.37***	192.52***	60.73***	23.45***	7.86**
BghiP	1.75^{ns}	38.05***	5.09*	0.20^{ns}	1.71^{ns}	0.11^{ns}	29.09***
IP	14.87***	15341.66***	4071.64***	7996.30***	475.38***	384.99***	850.85***
PAH4[a]	100.69***	33.49***	20.72***	3.93^{ns}	4.31*	0.18^{ns}	0.04^{ns}
Light PAHs[b]	5.78*	218.62***	14.04**	6.86*	8.19**	30.15***	8.14**
Heavy PAHs[c]	0.01^{ns}	469.49***	199.73***	516.87***	97.88***	21.32***	74.34***
Total PAHs	5.19*	219.21***	14.12***	6.79*	8.16**	30.20***	8.18**

ns = not significant. F x C = Fat content x Casing type; F x S = Fat content x Smoking procedures; C x S = Casing type x Smoking procedures; F x C x S = Fat content x Casing type x Smoking procedures

* $p < 0.05$.

** $p < 0.01$.

*** $p < 0.001$.

[a] PAH4 content was determined by the sum of BaA, CHR, BbFA and BaP.

[b] Light PAHs content was determined by the sum of ACL, NA, AC, FL, PHE, AN, FA, PY, BaA and CHR.

[c] Heavy PAHs content was determined by the sum of BbFA, BkFA, BaP, DBahA, BghiP and IP.

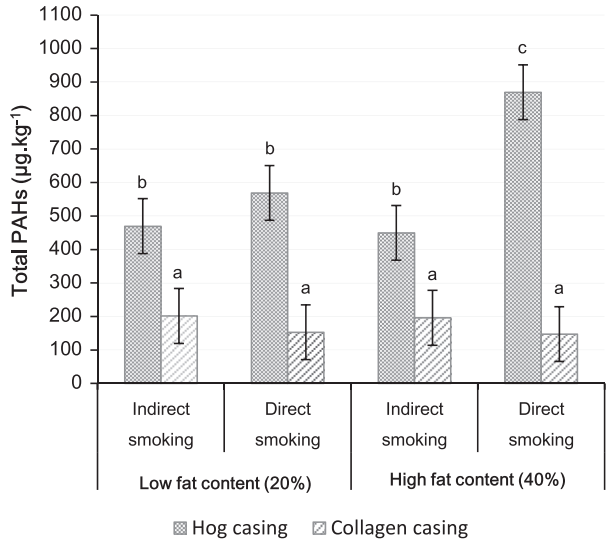

Fig. 2. Total PAHs content in whole product depending on fat content, casing type and smoking regime. Vertical bars denote 0,95 confidence intervals. Different letters represent significant differences (HSD test, $p = 0.05$).

fat contents resulted in different contamination levels depending on the used casing. For products stuffed in collagen casing, significant higher contamination levels were found in the 40% fat formulation, in opposition to what was observed for those manufactured with hog casing (Table 3).

Concerning the interaction between fat content and smoking regime ("F × S"), its effect was most significant regarding BbFA and IP where samples produced with direct smoking lead to the highest PAHs amounts, irrespective of fat content. When indirect smoking was used, significantly lower PAHs were generally found, with the fatter formulation showing higher contamination levels. Moreover, among all interactions, "F × S" interaction was the only one which significantly influenced the PAH4 marker (Table 2). For this particular PAHs group, no differences were detected as a result of direct/indirect smoking of leaner sausages. Nevertheless, the combination of 40% added fat and direct smoking systematically contributed to higher PAH4 contents.

From the evaluated two-way interactions (Table 2), the interaction between casing type and smoking regime ("C × S") was the less significant, even though 11 from the 16 studied compounds were significantly affected ($p < 0.05$), where IP and BbFA had the higher F values (384.99 and 222.98, respectively). In general, the amounts of detected low molecular weight PAHs were not affected by the different smoking options when collagen casing was used. It must also be pointed out that the highest PAHs contents were detected in products combining hog casing with direct smoke exposure.

Since samples were produced under the simultaneous combination of a specific fat content, casing type and smoking procedure, from the statistical stand point, the three-way interaction of factors, was also analyzed in the present study, which showed high significance values for most PAHs. Fig. 2 shows how the three-way interaction affected total PAHs contents in manufactured dry fermented sausages. When hog casing was used, significant higher ($p < 0.05$) PAHs amounts were detected, especially for sausages with 40% fat and smoked under a direct regime. For those products stuffed in synthetic casing instead, no significant differences ($p > 0.05$) were observed, irrespective of the fat content and smoking method. Similar results were obtained for IP, BaP, BbFA and BkFA in which higher F values were found.

Several studies reporting PAHs profiles in smoked meat products are available (Ciecierska and Obiedziński, 2007; Djinovic et al., 2008; Stumpe-Vīksna et al., 2008a; Roseiro et al., 2011; Santos et al., 2011) concerning multiple product types, manufacturing practices (wood type, smoking practices) and evaluated target compounds. Despite the variety of conditions assayed, all profiles were consistently similar regarding the prevalence of light PAHs over the heavy compounds. PAHs profiles obtained in this study

Table 3
Mean PAHs contents (μg kg^{-1}) of dry fermented sausage.

	Low fat content (20%)				High fat content (40%)				SE
	Hog casing		Collagen casing		Hog casing		Collagen casing		
	Indirect (n = 4)	Direct (n = 4)	Indirect (n = 4)	Direct (n = 4)	Indirect (n = 4)	Direct (n = 4)	Indirect (n = 4)	Direct (n = 4)	
ACL	177.45^{b,c}	272.46^{c,d}	130.67^{a,b}	53.08^{a}	146.45^{a,b}	342.20^{d}	85.44^{a,b}	42.67^{a}	25.86
NA	102.45^{b}	90.35^{a,b}	22.17^{a}	28.80^{a,b}	93.43^{a,b}	246.10^{c}	43.11^{a,b}	38.98^{a,b}	16.13
AC	57.26^{b}	64.16^{b,c}	15.83^{a}	23.48^{a}	76.95^{c}	117.77^{d}	22.36^{a}	20.23^{a}	2.79
FL	70.89^{b}	71.17^{b}	10.99^{a}	17.55^{a}	74.34^{b}	99.26^{c}	16.37^{a}	16.00^{a}	3.13
PHE	28.86^{d}	32.05^{d}	8.40^{a}	11.82^{b}	25.36^{c}	29.19^{d}	9.11^{a,b}	9.13^{a,b}	0.70
AN	3.32^{b}	4.18^{c}	0.75^{a}	1.16^{a}	3.33^{b}	5.68^{d}	0.82^{a}	0.74^{a}	0.14
FA	10.51^{c}	12.54^{d}	4.05^{a}	5.69^{a}	11.39^{c,d}	7.90^{b}	5.38^{a}	4.61^{a}	0.37
PY	12.01^{b,c}	13.79^{c}	4.40^{a}	6.23^{a}	10.08^{b}	10.73^{b}	5.75^{a}	5.40^{a}	0.42
BaA	1.99^{a}	2.18^{a}	3.49^{a}	3.30^{a}	6.30^{b,c}	7.92^{c}	5.73^{b}	7.40^{b,c}	0.39
CHR	3.88^{e}	4.52^{f}	0.57^{a}	1.10^{b}	1.58^{c,d}	1.89^{d}	1.01^{a,b}	1.27^{b,c}	0.10
BbFA	0.20^{d}	0.30^{g}	0.06^{a}	0.17^{b}	0.19^{c}	0.26^{f}	0.19^{c,d}	0.21^{e}	0.00
BkFA	0.05^{d}	0.07^{e}	0.00^{a}	0.04^{c}	0.03^{b}	0.06^{d}	0.04^{c}	0.04^{c}	0.00
BaP	0.32^{e}	0.31^{e}	0.09^{a}	0.21^{b}	0.19^{b}	0.27^{d}	0.23^{c}	0.24^{c}	0.00
DBahA	0.35^{c}	0.58^{d}	0.05^{a}	0.21^{b}	0.17^{b}	0.24^{b}	0.39^{c}	0.22^{b}	0.02
BghiP	0.06^{c,d}	0.05^{b,c,d}	0.00^{a}	0.05^{b,c,d}	0.05^{b,c,d}	0.08^{d}	0.04^{b,c}	0.02^{a,b}	0.01
IP	0.11^{f}	0.15^{g}	0.00^{a}	0.05^{b}	0.06^{c}	0.11^{f}	0.07^{e}	0.07^{d}	0.00
PAH4[A]	6.38^{b,c}	7.32^{c,d}	4.21^{a}	4.77^{a,b}	8.26^{c,d}	10.35^{e}	7.17^{c,d}	9.12^{d,e}	0.43
Light PAHs[B]	468.63^{b}	567.41^{b}	201.33^{a}	152.21^{a}	449.22^{b}	868.64^{c}	195.10^{a}	146.43^{a}	39.67
Heavy PAHs[C]	1.09^{d}	1.46^{e}	0.20^{a}	0.71^{b}	0.70^{b}	1.02^{c,d}	0.96^{c}	0.79^{b}	0.03

In the same line, means with different letters represent significantly different PAHs contents ($p < 0.05$). SE = standard error.
[A] PAH4 content was determined by the sum of BaA, CHR, BbFA and BaP.
[B] Light PAHs content was determined by the sum of ACL, NA, AC, FL, PHE, AN, FA, PY, BaA and CHR.
[C] Heavy PAHs content was determined by the sum of BbFA, BkFA, BaP, DBahA, BghiP and IP.

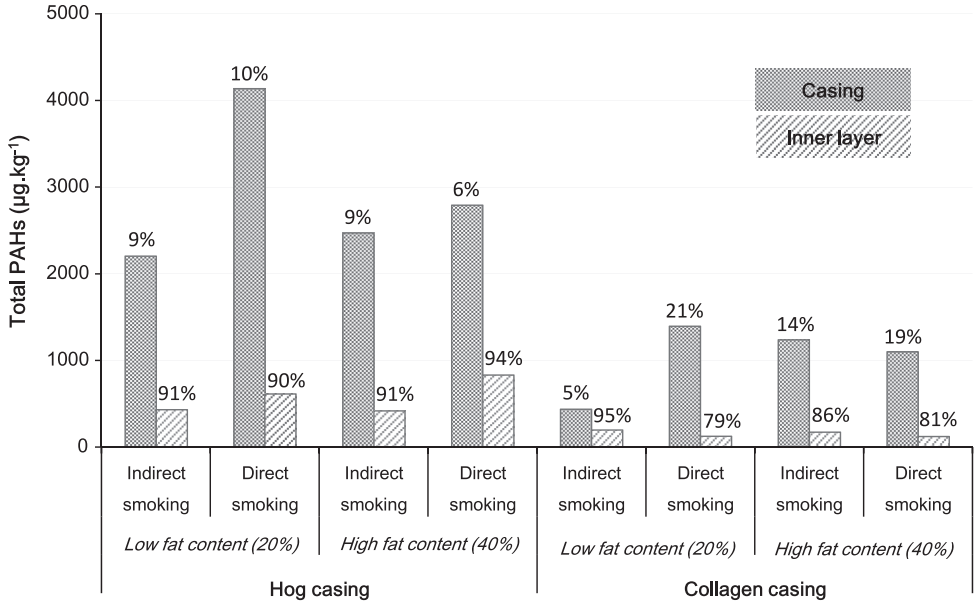

Fig. 3. Casing and inner layer total PAHs content and their relative contribution (expressed in %) for whole product contamination levels.

(Table 3), were similar to those reported by Santos et al. (2011) for Portuguese traditional dry fermented sausages from Alentejo (south of Portugal). In general, all sample PAHs profiles had in common the decrease in PAHs content as their molecular weight increased. As shown by Aurore et al. (2000), Guillén et al. (2000) and Stumpe-Vīksna et al. (2008b), prevalence of light PAHs can be attributed to the smoke composition it-self, independently of the wood used in combustion and smoking procedure (direct/indirect), since these low molecular weight compounds are usuallly found in higher amounts. In our study, PAHs up to 4 aromatic rings were found to represent >99% of the total detected PAHs, regardless of sample type which is in agreement with the findings of Gomes et al. (2009) and Santos et al. (2011). Among the found light PAHs in our study, those with 2 or 3 aromatic rings such as ACL, NA, AC, FL and PHE were always detected with their sum corresponding, at least, to 86% of the total PAHs.

Heavy PAHs were always found in smaller amounts (between 0.20 and 1.46 μg kg^{-1}) and never exceeded 0.5% of the total PAHs content, in whole product, irrespective of sample type (Table 3). Among the detected heavy compounds, DBahA and BaP emerged with the highest levels (0.58 and 0.32 μg kg^{-1}, respectively) and the later compound was about 15 times lower than the regulated limit (EC, 2011). For products with hog casing, those having high fat content showed lower heavy PAHs levels when compared to samples with lower fat content. This could be attributed to the migration effect from the casing to the inner layers, which is favored by high fat contents according to García-Falcón and Simal-Gándara (2005).

PAH4 levels ranged between 4.21 and 10.35 μg kg^{-1}, where the main contributors were BaA and CHR, both light compounds (Table 3). As expected, samples with 40% fat stuffed in hog casing and submitted to direct smoking procedure were those with the highest PAH4 contents which is, in fact, bellow the reference limit of 30 μg kg^{-1} (EC, 2011).

To evaluate the influence of the fat content, casing type and smoking procedures, processing conditions used in dry fermented sausages manufacture in the present study were not exactly the same, if compared with those usually applied in Portuguese meat industry, even though, results for BaP and PAH4 marker are in good agreement with those reported by Roseiro et al. (2011). Since total PAHs content seems to be too high in the present study, the risk associated with the consumption of these dry fermented sausages was also evaluated, following the approach based on the margin of exposure (MOE) as recommended by EFSA (2008). This methodology is based both in the compounds carcinogenic capacity and the consumers daily intake, where higher margins of exposure are indicative of low risk levels from the consumers health point of view. Considering a mean consumption of 25 g of dry fermented sausages per day, by a person weighting about 60 kg, that would represent a daily intake of 0.13 and 4.31 ng kg^{-1} bw day^{-1} for BaP and PAH4. According to our results, the determined MOE value is higher than 10,000 (considered as the reference value in EFSA (2008)). In face of this, manufactured products would be of low concern to consumers health.

4. Conclusions

The use of collagen casings significantly reduced the total PAHs content of the dry fermented sausages, contributing decisively to the related deposition/migration rate. Since most specifications for traditional meat products processing mandatorily include the use of hog casing, such option can still be used without major concern if indirect smoking regimes are applied. However, for safety reasons, the removal of any type of casing prior to consumption is recommended. According to 2008 EFSA recommendations, detected contamination levels in dry cured meat products manufactured according traditional processing procedures do not represent any considerable risk to consumers' health.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

The author Ana Gomes wishes to thank to the Foundation for Science and Technology (FCT) for the PhD grant (SFRH/BD/44255/2008). The authors also thank the funding provided by the European Commission through the SAFEFOODERA program, in the framework of the Research Project RISKFOODCONT (ERA-FOOD/0001/2008).

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