The Effect of Starter Cultures on the Portuguese Traditional Sausage "Paio do Alentejo" in Terms of Its Sensory and Textural Characteristics and Polycyclic Aromatic Hydrocarbons Profile

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Abstract

"Paio do Alentejo" is a Portuguese dry-cured sausage, made with meat from the Alentejano pig breed. The main aim of this study is to evaluate the benefits of the use of starter cultures on the quality of the sausage, mainly with regard to its sensory properties, rheological characteristics and PAH profile.

Three batches of the product were examined: S1-inoculated with a commercial starter comprising *Lactobacillus* spp., *Micrococcaceae* and yeasts; S2-inoculated with a starter comprising *Lactobacillus sakei* and *Staphylococcus xylosus*; C-the control batch, was not inoculated.

A sensory panel of 12 experts analysed samples in accordance with a descriptive analysis using a structured scale. A Texture Profile Analysis and 16 EPA priority PAHs for dry fermented sausages were performed.

Both inoculated batches were well received by the judges, no significant differences being noted between them. The use of S2 led to a slight improvement in terms of cohesiveness. However, the use of starter cultures was not found to influence PAH content.

Keywords: sensory properties, TPA, PAHs, Portuguese dry sausage, starter cultures

1. Introduction

In Mediterranean countries the consumption of dry-cured sausages is widespread, and in Portugal one of the most popular is "Paio". "Paio do Alentejo" is a commercial high-value product, manufactured using traditional ingredients and employing modern technology, and is usually produced without using starter cultures.

It is produced in the Alentejo region of south-east Portugal and is made with meat from the Alentejano pig breed, a Portuguese autochthonous breed produced in the Alentejo region, similar to the Iberian pig breed, whose meat is known for its high content of intra- and inter-muscular fat, which is rich in oleic acid (Elias & Carrascosa, 2010).

Starter cultures were first used in the early 20th century to improve food safety. Nowadays, they are used in order to achieve other kinds of improvement associated with, for example, sensorial, nutritional, safety and technological characteristics, and their use has been widely demonstrated to lead to benefits in terms of final product quality (Bover-Cid et al., 1999; Bover-Cid et al., 2001; Baka et al., 2011; Simion et al., 2014).

These cultures are composed mainly of *Lactobacillus* spp. and *Staphylococcus* spp., which have the capacity for improving sensorial and textural characteristics such as colour, flavour, taste, hardness, and cohesiveness, among others. Strains of these genera are used for inoculating minced-meat portions for sausage production in numbers varying from 10^5 to 10^8 cells of microorganisms per gram of meat. Yeasts and moulds are also used to improve sausage sensory quality, and the latter, due to their aerobic metabolism, are applied to the sausage skin or casing.

Texture profile analysis (TPA) is commonly used as an instrumental method of uni-axial compression and consists of compressing food material twice and quantifying the mechanical parameters using force-deformation

curves for food products (Szczesniak et al., 1963; Bourne, 1978; Foegedinga et al., 2003). According to Szczesniak (2002), one of the most important advantages of the use of this method is that it produces high correlations between some instrumental parameters and sensory ratings. Honikel (1997) tried to standardize methods for measuring meat texture, and strongly recommended the use of sensory evaluation in order to validate instrumental methods for determining tenderness. In a study examining fermented sausages by Herrero et al. (2007), TPA and physico-chemical measurements were performed, as well as a breaking strength by tensile test. The results obtained allowed these meat products to be grouped according to four different textural profiles. Yang et al. (2007) examined new preparation formulas for low-fat sausages with three different levels of added hydrated oatmeal and tofu. The aim of the study was to determine the effects of the type and level of texture-modifying agents on the physical and sensory properties of low-fat sausages. Rubio et al. (2007) carried out research on "salsichon" with high unsaturated fat content, vacuum packed under modified atmospheric conditions, analysing its microbiological, physico-chemical and sensory properties in order to evaluate quality changes during refrigeration storage. TPA was performed and the parameters determined from the force-time curves obtained were hardness, springiness, cohesiveness and chewiness. Wang et al. (2009) also performed TPA to determine the effects of phosphate level on water-holding capacity and texture of emulsion-type sausages during storage. Spaziani et al. (2009) examined low-acid sausages in order to characterize their physico-chemical, microbiological, and textural properties during ripening, also performing TPA; once again the parameters determined were: hardness, cohesiveness and adhesiveness. In the light of the results of all this previous research, TPA tests were used in the present study in order to identify differences in texture parameters, mainly in order to corroborate the sensory analysis results of previous studies.

Polycyclic aromatic hydrocarbons (PAHs) are organic compounds formed by the incomplete combustion of organic matter, environmental pollutants originating from countless natural processes and human activities. Contamination by PAHs deriving from intense thermal processes due to the direct deposition of PAHs from smoke occurs as a result of the incomplete combustion of different thermal agents. Because many of these are highly suspect as aetiological agents in human cancer, the chemical analysis of PAHs is of great environmental and toxicological importance (Lorenzo et al., 2011; Courter et al., 2008; Lin et al., 2008; Park et al., 2008). Among PAHs, benzo[a]pyrene (BaP) is used as a marker for the occurrence of carcinogenic PAHs (Lorenzo et al., 2011). In Europe, maximum tolerance levels for BaP in smoke condensates have been established (Commission Regulation EC no. 2065/2003) and are applied to several foodstuffs categories (EC nº 1881/2006). However, recent data has shown that BaP alone cannot be considered as a satisfactory indicator of PAHs occurrence in foods (EFSA, 2008). As a result, a new regulation (EC nº 835/2011) was introduced in September 2012 and provides for PAH4 marker contents, composed of BaP, benzo(a)anthracene (BaA), chrysene (CHR) and benzo(b)fluoranthene (BbFA), to be set at 30 µg kg^{-1} (EC, 2011). According to the European Food Safety Authority (EFSA, 2008), meat and meat products are one of the food categories contributing most to the dietary PAHs intake per day of European Union member state consumers. This demonstrates the important role of PAHs studies for all smoked food products, with a view to quantifying these compounds and identifying the factors which affect amounts occurring.

PAHs biodegradation depends on the nature and the chemical structure of the compound being degraded, environmental conditions, and the number and type of microorganisms involved. These compounds are biodegraded/biotransformed into less complex metabolites, and by means of mineralization into inorganic minerals: H_2O and CO_2, in aerobiosis, or CH_4 in anaerobiosis. The rate of biodegradation depends on several factors, such as chemical structure, their chemical portioning in the medium and their cellular transporting properties, besides environmental factors such as pH, temperature, oxygen availability, degree of acclimation, the availability of nutrients and the microbial population (Roseiro et al., 2010). A number of bacterial species are known to degrade PAHs and most of them are isolated from contaminated soil or sediments. *Alcaligenes* spp., *Enterobacter* spp., *Haemophilus* spp., *Pseudomonas aeruginosa*, *P. fluorescens*, *Sphingomonas* spp., *Neptunomonas* spp., *Rhodococcus* spp., *Bacillus* spp., *Paenibacillus* spp., and *Mycobacteria* spp. are some commonly studied bacteria. Also ligninolytic fungi have the capacity to degrade PAH. *Phanerochaete chrysosporium*, *Bjerkandera adusta*, and *Pleurotus ostreatus* are common PAH-degrading fungi (Kanaly & Harayama, 2000; Tian et al., 2008; Haritash & Kaushik, 2009; Arulazhagan & Vasudevan, 2011; Wang et al., 2012). Despite the fact that these microorganisms are used in soil or water decontamination, few studies relate PAHs degradation to *Lactobacillus acidophilus* (Wang et al., 2012) and *Staphylococcus warneri* (Moscoso, 2012). This enables the hypothesis to be formulated that some starters used in food production have the capacity for reducing PAHs.

A number of studies of "Paio do Alentejo" have been carried out in order to characterize this type of product,

which is known by a number of different names, in accordance with the region of origin of production (Elias et al., 2000; Elias et al., 2003; Roseiro et al., 2008; Roseiro et al., 2010; Elias & Carrascosa, 2010; Elias et al., 2011).

The main aim of this paper is to evaluate the effect of two starter cultures: a commercial starter culture, which has been previously tested by starter producer, and an experimental starter culture. Authors intent to evaluate the effect of both starter cultures on the sensory and textural properties and PAH levels of "Paio do Alentejo".

2. Materials and Methods

2.1 Sausage Technology and Sampling Procedures

"Paio do Alentejo", a Portuguese traditional cured sausage, is cylindrical shaped, with a diameter of 4 to 5 cm and a length of 25 to 30 cm. It is made with meat from the Alentejano pig breed, known for its high intra- and inter-muscular fat content. "Paio do Alentejo" was prepared at a traditional factory located in Alentejo region.

The production process involves mechanically mincing meat previously cut into cubes measuring approximately 2.5 cm and mixed with red pepper (*Capsicum annuum* L.) paste (6%), water (3%), garlic (*Allium sativum* L.) paste (1%), salt (0.7%), disodium diphosphate (0.03%), pentasodium triphosphate (0.03%), $NaNO_3$ (0.03%), KNO_3 (0.008%) and KNO_2 (0.007%). Red pepper and garlic pastes contain 17% of salt (NaCl).

One batch (S1) was inoculated with a commercial starter (TEXEL® ELSE BR) in accordance with the instructions of the starter producer, containing *Lactobacillus* spp., Micrococcaceae and yeasts; another batch (S2) was inoculated with an experimental starter containing *Lactobacillus sakei* (10^5 cells/g) and *Staphylococcus xylosus* (10^5 cells/g); a third batch (C), the control batch, was not inoculated.

The mixture of meat and other ingredients was stored under controlled conditions at 5 °C and 90% of relative humidity for 72 hours for ripening purposes. Then the meat was stuffed into natural casings made from the large intestines of pigs, measuring 50-55 mm in diameter.

The drying operation occurs in two phases: sausages are dried (1) in a smoking room (connecting directly with the exterior by chimney) for 48 hours [at a temperature of 18-24 °C and a relative humidity of 30-60%, with smoke generated by burning oak wood (*Quercus ilex*, L.)], (2) in special chambers under controlled conditions for 40 days (at a temperature of 9 °C and a relative humidity of 80-85%). After processing, sausages were used immediately for sensory, TPA, pH and a_w analysis, vacuum packed and stored deep frozen (-80 °C) until PAH analysis was carried out.

The sensory and textural properties (Texture Profile Analysis) of five samples from each different batch of (S1, S2 and C) were evaluated, while pH and a_w levels were also determined and PAHs quantified.

2.2 Sensory Evaluation

A panel of 12 qualified judges evaluated five sausage samples per batch. Sensory evaluation was carried out in a special room in accordance with the method described by Costell and Durán (1981 a, b, c, d). Whole sausages were cut into slices (3 mm thick) 30 minutes before analysis. A sample of five sausages per session was presented in random order. Three slices from each dry cured sausage were presented on small white plates denoted with a three-digit random number. A descriptive quantitative method was adopted, using a structured scale ranging from 0 (no sensation perceived) to 100 (maximum sensation perceived). The following attributes were considered: colour intensity, off colour, aroma intensity, off aroma, tenderness, succulence, flavour intensity, off flavour, salt perception and overall acceptability. For salt perception, the optimum value considered was 50 (values below 50 indicate low salt content while values above 50 indicate a high salt content). Judges rinsed their mouths out with neutral water and ate a cracker during samples evaluation.

2.3 Texture Profile Analysis

Texture Profile Analysis (TPA) using a cylindrical flat-ended plunger (with a diameter of 1.13 cm and an area of 1 cm^2) was performed using a Stable Micro System TA-Hdi (Stable Micro Systems, Godalming, England) in accordance with the methodology described by Honikel (1997), Caine et al. (2003) and Martinez et al. (2004). Tests were carried out at room temperature (20 °C ± 1 °C). The samples were previously cut into slices 1 cm thick, providing circular samples with a 4 to 5 cm diameter, which were compressed twice in two consecutive cycles of 50% compression with 5s intervals between cycles, while the plunger was actioned at a constant speed of 1 mm/s. Force/time curves were obtained in order to calculate the following parameters: hardness, adhesiveness, springiness, cohesiveness, gumminess, resilience, and chewiness.

2.4 pH and a_w

After removing the sausage casings, pH was determined in accordance with Norma Portuguesa NP - 3441 (1990) using a pH-meter (Crison 507, Barcelona, Spain). Regarding water activity (a_w), measurements were carried out using a hygrometer (Hygroskop Rotronic DT, Zurich, Switzerland) with a WA-40 probe at 25 °C.

2.5 Polycyclic Aromatic Hydrocarbons Profile (PAHs)

2.5.1 Standards and Reagents

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

2.5.2 PAH Extraction

As regards the casing and the sausage meat/fat mixture, PAH extraction was performed in accordance with indications provided by Santos et al. (2011). Samples were homogenized in a Grindomix (GM 200 Retsch, Haan, Germany) and 10 g was saponified under reflux in the presence of a mixture of potassium hydroxide, water and methanol. 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 rotatory 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 a chromatographic system for quantification.

2.5.3 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 carried out in accordance with indications provided by Santos et al. (2011).

Limits of detection (LOD), quantification (LOQ) and recovery were described by Gomes et al. (2013). The sum of the final PAH content in samples was regarded as total PAH content of the whole product.

2.6 Statistical Analysis

For the purpose of statistical data processing, an ANOVA was performed, using the Statistica 5.1 program, and means were compared using the HSD Tukey test. Principal Components Analysis (PCA) was also used.

3. Results

3.1 Sensory Analysis

ANOVA for sensory results evidenced no significant differences for any attribute ($p > 0.05$). Overall acceptability values were very similar for sausages from the three batches while the highest value was recorded for Batch C (Table 1). In this batch, colour intensity, aroma intensity and flavour intensity were the lowest out of the three batches, suggesting that the panellists showed a preference for a rather bland product. The use of starter cultures in sausages to improve colour, aroma and flavours intensities is widespread, and while this is widely recognized it was not appreciated by the panellists, probably due to the high quality of "Paio Alentejano" produced for this research. Batch S2 produced the lowest overall acceptability but highest scores for aroma intensity, succulence and salt perception. The production of some microorganism metabolites, among them lactic acid bacteria, may provide an unusual flavour; however, this is not the same as off flavour.

Table 1. Arithmetic Mean values (from 0 to 100) and Standard Deviation for sensory variables for different batches: C (control), S1 (commercial starter culture) and S2 (experimental starter culture)

Batch	Colour intensity	Off Colour	Aroma intensity	Off Aroma	Tenderness	Succulence	Flavour intensity	Off Flavour	Salt Perception	Overall Acceptability
C	66.8 ±12.2	0.0	69.7 ±10.0	0.0	55.4 ±8.8	68.6 ±8.0	72.4 ±9.2	0.0	56.1 ±10.7	73.2 ±7.2
S1	71.1 ±14.1	0.0	71.0 ±11.2	0.0	54.1 ±5.2	67.1 ±9.9	73.5 ±9.9	0.0	58.1 ±9.7	72.9 ±10.4
S2	69.9 ±15.0	0.0	72.0 ±11.4	0.0	51.4 ±8.4	71.9 ±8.5	73.1 ±10.1	0.0	59.7 ±10.8	71.8 ±10.6
F value	0.695		0.185		1.864	0.685	0.085		0.818	0.151
p value	0.502		0.832		0.162	0.507	0.919		0.445	0.860

3.2 TPA

Both the scores awarded by the panellists and instrumental measurements were very similar for three batches (S1, S2 and C).

In accordance with ANOVA for TPA results, the only significant difference was obtained for cohesiveness ($p < 0.05$), which was higher for Batches S1 and S2 as compared with Batch C (Table 2). Here, starter cultures contributed towards an increase in the linking forces between meat portions in inoculated sausages. From a practical point of view, this is an extremely important feature, which could justify the use of starters, even with high-quality products. Besides this, these sausages produced with starters were more tender (hardness 41.34 N and 41.99 N) than those of control batch (45.07 N). The results obtained for both inoculated batches were generally very similar, despite their different composition: a commercial starter and an experimental starter, which has not yet been used for commercial purposes.

Table 2. Arithmetic Mean values and Standard Deviation for TPA variables for different batches: C (control), S1 (commercial starter culture) and S2 (experimental starter culture)

Batch	Hardness (N)	Adhesiveness (N x s)	Springiness (mm)	Cohesiveness	Gumminess (N)	Resilience	Chewiness (N x mm)
C	45.07 ±12.27	-2.61 ±1.10	0.85 ±0.06	4.31[b] ±0.45	28.59 ±8.02	0.17 ±0.03	24.58 ±7.84
S1	41.34 ±8.59	-3.46 ±1.09	0.88 ±0.07	4.75[a] ±0.34	26.59 ±4.96	0.16 ±0.02	23.47 ±5.06
S2	41.99 ±12.96	-3.24 ±1.39	0.84 ±0.06	4.63[a] ±0.54	26.41 ±6.85	0.16 ±0.03	22.26 ±6.52
F value	0.455	2.018	1.832	3.748	0.484	1.736	0.467
p value	0.637	0.146	0.173	0.032	0.620	0.189	0.630

Within the same parameter (column), means with a different superscript are significantly different ($p < 0.05$).

Onega et al. (2001) in a study of raw and cooked meat also found that hardness, springiness and chewiness obtained by means of TPA tests showed a high degree of correlation with sensory parameters.

Sometimes this degree of coherence has not been evident, such as in the study carried out by Caceres et al. (2006), who reported that although statistical analysis showed significant differences, changes are not reflected in sensory analysis for batches of sausages with different fat levels and different amounts of added calcium.

With regard to PCA analysis (see Figure 1 and Table 3), Factor 1 accounted for 31% of the variance of variables and established the association between succulence, deriving from sensory evaluation, and adhesiveness,

obtained from TPA, both of which are shown on one side of the Factor 1 axis and show a positive factor-variable correlation (see Table 4). This may be related to a high degree of attractiveness of the surface or skin of "Paio do Alentejo" and the consequent positive effect on the judges' palate and teeth, which may be reflected in a high level of saliva production which thus may lead to the high scores recorded for succulence. Factor 2 accounted for 20% of results, and cohesiveness deriving from TPA is related to succulence deriving from sensory evaluation. Once more, succulence, as evaluated by the judges, correlates positively with a textural parameter. PCA projection of variables revealed the very close relationship between hardness, as evaluated by the panellists, on the one hand, and TPA on the other hand, which are both located in the same quadrant; this may provide an indication of the excellence of the performance of the panel, being able to distinguish even small changes in hardness. Ruiz de Huidobro et al. (2005) also found that with regard to sensory properties such as hardness, TPA was a better predictor than other instrumental methods.
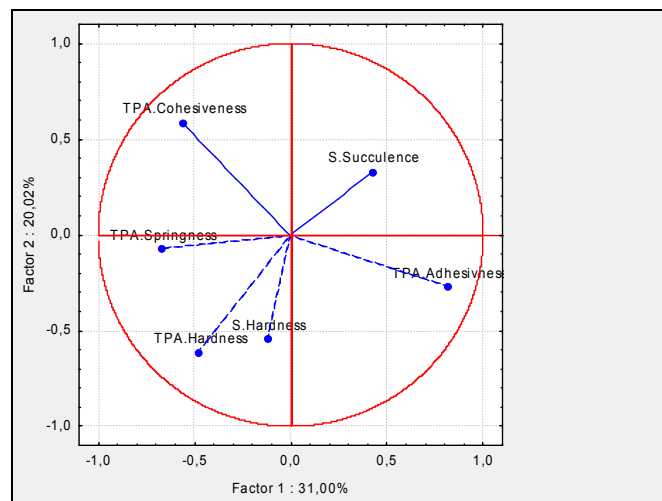
Figure 1. Principal Component Analysis. Projection of variables on the factor plane, considering 2 factors

Table 3. Principal Component Analysis. Eigenvalues of correlation matrix and related statistics

Factor	Eigenvalue	% Total
1	1.860	31.004
2	1.201	20.020
3	1.036	17.269
4	0.931	15.518
5	0.553	9.223
6	0.418	6.966

Table 4. Principal Component Analysis. Factor-variable correlations (factor loadings), based on correlations

	Factor 1	Factor 2	Factor 3
Sensory Hardness	-0.124	-0.543	0.560
Sensory Succulence	0.425	0.330	-0.520
TPA Hardness	-0.478	-0.613	-0.411
TPA Adhesiveness	0.812	-0.270	0.145
TPA Springiness	-0.676	-0.073	-0.307
TPA Cohesiveness	-0.565	0.586	0.410

Hardness, chewiness and springiness are extremely useful parameters for the assessment of meat texture (Ruiz

de Huidobro et al., 2001). González-Fernández et al. (2006) prepared four batches of Spanish chorizo; one was a control batch while the other three batches were inoculated with *L. sakei* K29, *Pediococcus* sp. P22 and *Pediococcus* sp. P208, respectively; the textural parameters examined were hardness, springiness, cohesiveness and chewiness, and for all parameters the lowest value was that of the control batch. Similar results were obtained by Marcos et al. (2007) for Spanish fuet (Spanish thin cured-dry pork sausage) and chorizo. With the exception of cohesiveness, the results of the present study are different, as hardness and chewiness were higher for Batch C than Batches S1 and S2; in addition, springiness for Batch C was similar to that of Batch S2 but lower than that of Batch S1 (see Table 2).

3.3 pH and a_w

The results of an analysis of variance of pH and a_w values do not show significant differences between batches, while mean values for these variables are very similar (see Table 5). In fact, the use of the two starter cultures did not influence either pH or a_w. pH mean varied from 5.23 (Batch C) to 5.26 (Batch S2), while a_w ranged from 0.881 (Batch S1) to 0.884 (Batches C and S2). Once again, the use of the two starters did not appear to have any influence on the characteristics of "Paio do Alentejo". Elias and Carrascosa (2010) studied "Paio do Alentejo" produced at two manufacturing plants and obtained a pH mean value of 5.45 and an a_w mean value of 0.821 for non-smoked products, and a pH mean value of 5.74 and an a_w mean value of 0.818 for smoked products. These values are comparable with those obtained in the present study as lower a_w values reflect a greater degree of curing, while the relatively higher pH values recorded by Elias and Carrascosa (2010) may result from proteolytic reactions occurring in the final stage of curing. Similar pH and a_w values for "Paio do Alentejo" in the present study were comparable with those recorded by Elias et al. (2011) and Elias and Carrascosa (2013).

Table 5. Arithmetic Mean values and Standard Deviation for pH and a_w variables for different batches: C (control), S1 (commercial starter culture) and S2 (experimental starter culture)

Batch	pH	a_w
C	5.23	0.884
	±0.05	±0.008
S1	5.25	0.881
	±0.10	±0.018
S2	5.26	0.884
	±0.08	±0.021
F value	1.912	1.748
p value	0.161	0.187

3.4 PAH

Regarding the PAH analysis of variance of the results of the present study, no significant differences were found for any PAH ($p > 0.05$) for the three inoculation types. In accordance with ANOVA, arithmetic mean values for PAHs detected for the three batches are statistically comparable (see Table 6). Nevertheless, Batch S1 showed the lowest concentrations with regard to the majority of PAHs. Total PAHs, Light PAH, Heavy PAH and PAH4 levels were also lower for Batch S1.

Light PAHs include ACL, NA, AC, FL, PHE, FA, PY, BaA and CHR, while Heavy PAHs include BbFA, BaP, BkFA, DbahA, BghiP, IP, and PAH4 includes ACL, NA, BbFA and BaP.

The prevalence of Light PAHs over heavy compounds for different smoked meat products has been reported on several occasions by different authors (Ciecierska & Obiedziński, 2007; Djinovic et al., 2008; Stumpe-Vīksna et al., 2008; Roseiro et al., 2011; Santos et al., 2011). However, the concentration of each is variable.

The five major compounds found in the present study are AC, FL, PHE, NA and ACL; they are light PAHs with 2 or 3 aromatic rings, representing more than 83% of total PAHs. The results of this study agree with those obtained by Gomes et al. (2013). The total amount of heavy PAHs accounts for 0.65% of total PAHs (Batch C), being the lowest value recorded, and 0.72% (Batch S1), the highest value recorded. The highest concentration of heavy PAHs was 2.03 μg kg^{-1} (DbahA, Batch S2) and the lowest concentration was 0.66 μg kg^{-1} (BkFA, Batch S1), while concentrations of each heavy PAH for each batch were very similar. BaP levels were 1.00 μg kg^{-1}

(Batch S1), 1.03 μg kg^{-1} (Batch C) and 1.06 μg kg^{-1} (Batch S2). The lowest value recorded for PAH4 was 48.80 μg kg^{-1} (Batch S1) and the highest was 55.64 μg kg^{-1} (Batch C). These values were higher than those obtained by Gomes et al. (2013) for one type of Portuguese traditional sausage. These authors obtained values ranging from 4.21 to 10.35 μg kg^{-1} and the main contributors to this were BaA and CHR, both light compounds, as is the case with the present study. While the results reported by Gomes et al. (2013) were obtained using sausages without casings, in the present study samples were made up of products both with and without casings, which accounts for high PAHs levels (Roseiro et al., 2011; Santos et al., 2011). According to EFSA (2008), even if the casings of "Paio do Alentejo" are consumed (which does not normally occur), PAHs levels represent an irrelevant hazard.

Table 6. Arithmetic Mean values for the PAH content (μg kg^{-1}) for the different batches: C (control), S1 (commercial starter culture) and S2 (experimental starter culture)

PAH / Bath	C	S1	S2	F value	p value
ACL	91.55	81.86	78.75	0.174	0.844
NA	178.57	128.89	182.07	0.720	0.524
AC	220.97	200.09	216.73	0.160	0.856
FL	204.21	173.01	213.47	0.359	0.713
PHE	186.32	184.27	200.51	0.059	0.943
AN	57.68	39.05	51.84	1.158	0.376
FA	29.45	33.60	30.97	0.062	0.941
PY	26.14	24.74	24.32	0.030	0.970
BaA	23.94	21.44	25.58	0.187	0.834
CHR	29.27	25.09	23.08	0.315	0.741
BbFA	1.40	1.27	1.50	0.240	0.794
BaP	1.03	1.00	1.06	0.054	0.948
BkFA	0.67	0.66	0.75	0.238	0.795
DBahA	1.88	1.87	2.03	0.335	0.728
BghiP	1.86	1.80	1.95	0.449	0.658
IP	N.D.	N.D.	N.D.		
PAH4[A]	55.64	48.80	51.22	0.173	0.845
Light PAHs[B]	827.12	711.95	830.59	0.287	0.761
Heavy PAHs[C]	6.84	6.60	7.29	0.272	0.771
Total PAHs	1 054.93	918.64	1 054.61	0.270	0.772

N.D. - not detected.; [A]PAH4 content was determined by adding BaA, CHR, BbFA and BaP. [B]Light PAHs content was determined by adding ACL, NA, AC, FL, PHE, AN, FA, PY, BaA and CHR. [C]Heavy PAHs content was determined by adding BbFA, BkFA, BaP, DBahA, BghiP and IP.

Despite the lack of significant differences between batches for all PAHs, samples inoculated with starter S1 show lower PAH values, except for ACL and FA (see Table 6). These results may be related to the benefit of the use of starter S1 in order to decrease PAH production.

5. Conclusions

The addition of starters to "Paio do Alentejo" produced no significant improvement in sensory or rheological properties (except for cohesiveness), or PAH levels.

Total values for PAHs, PAH4, light PAHs and heavy PAHs were smaller than those recorded in other studies of traditional Portuguese sausages (Roseiro et al., 2011; Santos et al., 2011). This is due to the relatively small duration of exposure to smoke (48 hours) to which such products are subject. However, considering the fact that

the manufacturing process for the sausages examined in the present study involved stuffing meat in natural casings with a high fat content, the casing accounts for the majority of PAH values. Because casings are not usually consumed, real PAH values in terms of human consumption are lower than those reported in the present paper.

In this study, no differences were found between the three batches. This may be accounted by the presence of interesting technological microorganisms, such as lactic acid bacteria and Micrococcaceae, in the usual house flora. Usually, such flora is well adapted to the environmental conditions of the respective habitat and, for this reason, assumes a dominant role, thus reducing the impact of added microorganisms.

The lack of positive effects noted with regard to such starters is probably due to the high quality of "Paio do Alentejo" produced at the meat manufacturing plant, which is evident even without the use of starters: it should be noted that all batches received high scores from the panellists.

Starter cultures were initially developed and used for improving the food safety of meat products, by their presence, avoiding pathogens growth, and producing metabolites such organic acids, or bacteriocins, among others. Later, they were used for enhancing other aspects of quality such as sensory, textural and technological characteristics. Thus, any advantages of using starter cultures with products characterised by a very high level of quality are not immediately evident. Nevertheless, the use of starter cultures with such products may be recommended in order to combat the possible negative impacts of unforeseeable technological problems, thus providing an additional guarantee of the quality of final products.

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