

Animal Health, Biosafety and Food Safety

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SUMMARY

ADDITIONAL KEYWORDS

Pork Meat.
Traditional Sausages.
Chemical hazards.
Biological hazards.

Pork meat and processed pork meat products may carry some hazards that threaten the consumer's health. These hazards are related with animal health or with the way in which the raw materials are manipulated during slaughtering and manufacturing until the consumption. Substances having anabolic effects, the residues of veterinary drugs and chemical contaminants, some of them with origin in animal production but others produced during the transformation processes should be considered among chemical hazards. Concerning biological hazards, we can mention some parasites like *Toxoplasma gondii*, *Trichinella spiralis*, *Taenia solium* and also some bacteria like *Salmonella* spp., *Campylobacter* spp., *Yersinia enterocolitica* and *Listeria monocytogenes*. The physicochemical characteristics of fresh meat further facilitate the growth of various microorganisms, other than those mentioned above, which turns it into a highly perishable product. Several transformation processes of the raw material, provide different food products to consumers and constitute ways to increase its shelf-life. Drying, fermentation and cure are among these processing methods. In Portugal there is a wide variety of processed meat products, such as *Chouriço*, *Paio*, *Presunto*, *Salsichão* and *Catalão* many of them manufactured according to traditional techniques of their regions of origin. Some of these products have been studied at the Universidade de Évora. Some results, which show the favourable effect of a range of transformation processes in controlling some of the mentioned biological hazards, will be presented.

Saúde Animal, Biossegurança e Segurança dos alimentos

RESUMO

PALABRAS CLAVE ADICIONALES

Carne de porco.
Enchidos tradicionais.
Perigos químicos.
Perigos biológicos.

Alguns perigos que podem ameaçar a saúde dos consumidores de carne de porco e de produtos processados estão relacionados com a saúde animal ou com a manipulação da matéria-prima desde o abate dos animais, a transformação e até ao consumo. Entre os perigos químicos consideram-se as substâncias com efeitos anabólicos, os resíduos de substâncias medicamentosas e os contaminantes químicos, alguns com origem na produção animal ou produzidos durante o processamento. No que se refere aos perigos biológicos, podem encontrar-se alguns parasitas como *Toxoplasma gondii*, *Trichinella spiralis* e *Taenia solium* e também bactérias como *Salmonella* spp., *Campylobacter* spp., *Yersinia enterocolitica*, *Listeria monocytogenes*. As características físico-químicas da carne fresca facilitam o crescimento de diversos microrganismos, para além dos mencionados anteriormente, o que a torna altamente perecível. Os diferentes processos de transformação desta matéria-prima, não só permitem obter diferentes alimentos para o consumidor como constituem formas de aumentar a sua vida útil. Entre os processos de transformação incluem-se a secagem, a fermentação e a cura. Em Portugal há uma grande variedade de produtos cárneos tais como *Chouriço*, *Paio*, *Presunto*, *Salsichão* e *Catalão*, muitos dos quais são fabricados segundo as técnicas tradicionais das diferentes regiões de onde são originários. Alguns destes produtos têm sido estudados na Universidade de Évora. Apresentaremos alguns resultados que mostram o efeito favorável de vários processos de transformação no controlo de alguns dos perigos biológicos mencionados.

INFORMATION

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INTRODUCTION

Some hazards that may threaten the health of consumers of pork meat and processed products are related to animal health or with the way raw material is handled during collection, processing and distribution until consumption. In Portugal, according to INE (2016), about 44.7 kg/person/year of pork meat was

consumed in 2015, and in the same year about 132 000 tons of processed products were obtained. In Europe, pork meat consumption reached 40.9 kg/person/year (AHDB, 2016). The consumed amount may represent a high risk to the health of consumers due to the high exposure to chemical and microbiological hazards. Both may have origin in the animal itself, but they can also

reach the meat through environmental contamination and by improper handling practices.

CHEMICAL HAZARDS

Different chemical hazards may be present in pork and processed products. Some are originated in animal production, such as the residues of pharmacological active substances and others come from processing and transformation of raw materials in different pork meat products.

There are some substances whose use in animals intended for human consumption is forbidden, as is the case of steroids, β -agonists, tireostatics, nitrofurans and chloramphenicol. There are also substances whose use is allowed. For some of them, a maximum limit was established for the presence of their residues (MLRs) in the edible part of carcasses, as is the case of antibiotics, antiparasitics and nonsteroidal anti-inflammatories. For other substances, there was no need to establish MLRs, given their safety. This group includes some inorganic compounds, such as calcium acetate and copper sulphate; organic compounds, such as caffeine, theobromine, theophylline and vitamins; a wide variety of substances considered harmless, as eucalyptol, guaiacol and several amino acids; several substances used as homeopathic veterinary products, such as *Calendula officinalis* and *Hypericum perforatum*; and various substances of plant origin, such as aloe, mint and rosemary.

Regarding Portugal, pork fresh meat data from the 2011 report of the National Plan for Residues Detection, (the last one available online) revealed that in 1921 pig samples analysed, only in three samples taken in the slaughterhouse were detected residues of antimicrobial substances (DGAV, 2011). Concerning manufactured products data, the 2013 report of the National Plan of Sampling revealed that among the 33 analysed samples, sulphites were detected in two samples of ground meat, without mentioning the meat species (ASAE, 2013).

Polycyclic aromatic hydrocarbons (PAHs) are organic compounds formed by incomplete combustion of organic matter. They can have genotoxic and carcinogenic effects. Food can be contaminated from the environment, by domestic or industrial transformation

processes (EFSA, 2008). Studying the effect of starter cultures used in the manufacture of "Paio do Alentejo", concerning the PAHs' profile, Elias *et al.* (2014a) detected HAP4 values (sum of benzo (a) pyrene, benzo (a) anthracene, benzo (b) fluoranthene and chrysene) between 48.8 and 55.6 $\mu\text{g}/\text{kg}$. These values are slightly higher than the value established in Regulation (EC) No. 1881/2006, which indicates that the MRLs for these compounds are 30 $\mu\text{g}/\text{kg}$. This regulation established the value of 5.0 $\mu\text{g}/\text{kg}$ for benzo (a) pyrene (BaP). In this study, values between 1.0 and 1.06 $\mu\text{g}/\text{kg}$ were found. This content was detected in samples in which the sausages' casings were kept. Generally, they are removed prior to ingestion by consumers. Although casings represent only 1 to 2% of the total sausage mass, the level of PAHs found in the casings is much higher than that of the batter (Gomes *et al.* 2013). This fact can contribute to minimize the exposure of the consumer to this hazard in this type of product.

Biogenic amines result from the decarboxylase activity of microorganisms on the amino acids constituting the proteins present in the products. It depends on the interaction of several factors and, sometimes, can cause adverse effects to the health of consumers, as the case of vasoactive amines (phenylethylamine, tryptamine, tyramine and histamine). In some traditional Portuguese sausages studied by the team of the *Universidade de Évora*, aiming the production of sausages with reduced salt content, the content in biogenic amines is listed in **Table I**. The high value of vasoactive amines is due mainly to the tryptamine and tyramine levels (data not shown). The values of putrescine and cadaverine are also high. Although not having any detrimental effect themselves, they can potentiate the toxic effect of histamine. In the present case, this effect can be considered negligible, because histamine was neither detected or its content was very low. For Eerola *et al.* (1998), the values of vasoactive amines in products should be less than 200 mg/kg. This value was exceeded in the following products: *Salsichão* (with 3 and 6% salt) and *Paio preto* and *Catalão* with 3% salt. Regarding histamine, an amine with major effects on the consumers' organism, the values found were low. There is no legislation on this parameter for this kind of products, but Stadnyk & Dolatowski (2010) consider 100 mg/kg as the safety limit value. Comparing the products made with reduced salt content with those produced with

Table I. Content of vasoactive amines (mg/kg) found in several traditional sausages, with reduced (3%) and traditional (6%) salt content (Teor de aminos vaso-activas (mg/Kg) detectadas em diferentes enchidos tradicionais fabricados com teores de sal reduzido (3%) e tradicional (6%).)

Product	Salsichão		Catalão		Chouriço preto		Paio preto	
	3%	6%	3%	6%	3%	6%	3%	6%
Vasoactive amines	233.3 ^b ±23.3	251.5 ^b ±51.8	326.09 ^c ±9.5	166.39 ^a ±22.8	159.7 ^b ±20.7	19.31 ^a ±21.6	292.7 ^a ±24.9	164.15 ^b ±9.5
Histamine	15.02 ^d ±6.5	9.04 ^{bc} ±0.5	ND ^a	5.86 ^b ±1.1	ND	ND	28.87 ^a ±6.3	ND
Putrescine	24.57 ^d ±6.3	ND ^a	8.31 ^{bc} ±1.5	4.43 ^{ab} ±2.4	180.11 ^b ±10.9	265.36 ^c ±16.0	142.31 ^b ±68.3	81.01 ^a ±24.3
Cadaverine	577.87 ^c ±44.4	83.34 ^a ±41.6	178.37 ^b ±15.3	103.36 ^a ±10.8	364.83 ^c ±20.7	ND	270.80 ^b ±60.5	47.61 ^a ±10.9

ND: not detected

(Results of *Salsichão* and *Catalão* adapted from Laranjo *et al.* 2016a; Results of *Chouriço Preto* and *Paio Preto* adapted from Laranjo *et al.* 2017)

Table II. Microorganisms present in different traditional sausages, with reduced (3%) and traditional (6%) salt content (cfu/g) (Grupos microbianos presentes em diferentes enchidos tradicionais fabricados com teores de sal reduzido (3%) e tradicional (6%) (ufc/g).

Product	<i>Salsichão</i>		<i>Catalão</i>		<i>Chouriço Preto</i>		<i>Paio Preto</i>	
	3%	6%	3%	6%	3%	6%	3%	6%
Mesophiles	5.47±0.89	6.21±0.38	6.03±0.08	4.92±0.31	6.22 ^a ±0.19	7.59 ^{bc} ±0.71	8.21 ^c ±0.21	6.42 ^a ±0.12
Lactic acid bacteria	7.82 ^c ±0.09	7.44 ^{bc} ±0.16	7.5 ^{bc} ±0.07	5.23 ^a ±0.28	7.82±0.15	8.24±0.37	9.48±0.19	6.75±0.36
Enterobacteria	3.46 ^{ab} ±0.71	3.96 ^{bc} ±0.0	1.74 ^a ±0.02	0.43 ^a ±0.75	2.03±0.58	2.71±0.14	1.39±1.19	Nd
Enterococci	4.07 ^{bc} ±1.83	2.96 ^{abc} ±0.0	1.93 ^a ±1.08	2.22 ^{abc} ±0.05	1.73±0.15	1.27±0.12	2.74±0.16	1.68±0.33
Staphylococci	2.29 ^b ±0.15	2.96 ^{bc} ±0.0	Nd ^a	4.21 ^c ±0.15	3.26±0.24	4.49±0.24	3.29±0.19	4.04±0.27
Yeasts	3.11 ^a ±0.21	4.45 ^{bc} ±0.39	3.25 ^{ab} ±0.27	4.29 ^{bc} ±0.22	3.51±0.21	4.61±1.44	4.44±0.75	4.93±0.25

ND: Not detected

Different letters in the same row denotes statistically significant differences (p<0.05). No letters mean the differences between treatments were not significant.

(Results of *Salsichão* and *Catalão* adapted from Laranjo et al. 2016a; Results of *Chouriço Preto* and *Paio Preto* adapted from Laranjo et al. 2017)

regular salt content, we also noted that the first ones revealed higher content in biogenic amines (Table I). The reduced salt content allowed microbial populations, mainly lactic acid bacteria, to grow, thus increasing the decarboxylase activity (Table II). The results presented by Laranjo et al. (2016a; 2017), revealed that amines content generally increased over the ripening period. This increase is due to the growth of some microbial groups, in particular of lactic acid bacteria, many of which develop decarboxylase activity.

BIOLOGICAL HAZARDS

Considering biological hazards that may be present in pork meat, some parasites, such as *Toxoplasma gondii*, *Trichinella spiralis* and *Cysticercus cellulosae* may represent a threat to the consumers' health.

T. gondii can infect humans by ingestion of their cysts present in pork meat. Usually the infection is not due to food ingestion but instead by ingestion of the oocysts spread by the faeces of cats, route of infection that also occurs in pigs.

T. spiralis can infect human consumers via ingestion of cystic larvae present in raw meat. This represents a specific risk, which determines the total disapproval of the meat of infected animals that is considered unsuitable for consumption (Reg. (EC) No. 854/2004).

EFSA (2011) considers these two hazards relevant in Europe, being of 0.0004% the average prevalence of *T. spiralis* in fresh pork. EFSA suggests that the assessment of these hazards must be based on the use of Food Chain Information (FCI), on pig health status. The aim of this was only to submit to additional measures of *post-mortem* inspection, the carcasses from high risk herds. The carcasses of pig batches considered of low risk of *T. gondii* and *T. spiralis* can be marketed after the slaughter and chilling, without application of additional control measures. There are few effective methods to eliminate these meat parasites, in addition to the thermal treatment either by heat or by cold. The proposed measures to be applied in slaughterhouses may, at least in part, improve the safety of this raw material. However they can limit the use of meat from free-range animals, as it is the case of those used in some traditional sausages.

According to the report published by EFSA (2016), in Europe only 3.7% of the pigs evaluated were seropositive in relation to *T. gondii*. They were distributed in only 3 Member States (not in Portugal) and in a non-Member State of the European Union. However, in Portugal, the seroprevalence of *T. gondii* in humans was 22% in 2013, similar to values found in other Mediterranean countries like Spain, Italy and Greece (Gargatè et al. 2016). As for *T. spiralis* in Europe in 2015, more than 171 million of pigs were evaluated and only 106 animals were positive, which corresponds to a rate of

Table III. Results obtained by the Food Inspection Plan (PIGA) for pork meat and meat products (Resultados obtidos pelo Plano de Inspeção dos Géneros Alimentícios (PIGA) para carne de porco e produtos cárneos de porco).

Product	Analysed samples	<i>Salmonella</i> spp.	<i>Campylobacter</i> spp.	<i>Listeria monocytogenes</i>
Pork meat	66	6	4	0
Minced meat and meat preparations intended to be eaten cooked.	51	6	0	0
Meat products	223	9	0	10
TOTAL	340	21/340	4/116	10/156

Adapted from DGAV (2015)

0.6 per million (0.00006%). Most of the positive (about 82%) were found in four countries, that not include Portugal, in animals reared under not controlled conditions. 156 cases of trichinellosis were confirmed in humans (EFSA, 2016). This parasitosis is not diagnosed in humans in Portugal since 1966 (Ferreira et al. 2014). However, the same authors state that probably trichinellosis has been under-diagnosed. The study they conducted in two groups of people with high exposure to pork and wild boar meat not subject to sanitary inspection revealed the presence of antibodies anti-*T. spiralis* in about 4.4% of assessed individuals.

Taenia solium/Cysticercus cellulosae is currently not present in Europe. However, it should continue to be monitored, since it can re-emerge (EFSA, 2011). This monitoring is done through visual inspection of pig carcasses in slaughterhouses upon *post-mortem* inspection (Reg. (EC) No. 854/2004).

Concerning microbial hazards, there are few data on the incidence of pathogenic microorganisms in meat in Portugal (Xavier et al. 2014). These authors suggest that the average incidence of *Salmonella* spp. is 12.6% and of *Yersinia enterocolitica* is 6.8%, in pork meat. In meat products, the incidence of *Salmonella* spp. and *Listeria monocytogenes* is 9.7% and 8.8%, respectively. These values are lower in products intended to be eaten raw, comparing with the ones found in products intended to be cooked.

Data revealed by the 2015 Food Inspection Plan (PIGA) (DGAV, 2015) are presented in **Table III**, showing the presence of *Salmonella* spp. either in fresh meat or in other products. Probably this contamination comes from the slaughterhouse, during slaughter operations and it goes on over the various processes of meat preparation and transformation. It must be pointed out that detection of *Campylobacter* spp. was performed only in 116 samples. The ten positive samples for *L. monocytogenes* were found among 156 sam-

ples: seven were in ready-to-eat products and three were in products to be eaten cooked (DGAV, 2015).

When we analysed *Salsichão* and *Catalão* (Laranjo et al., 2016a) and *Chouriço preto* and *Paio preto* (Laranjo et al. 2015, 2017) manufactured with regular and reduced salt content, *Salmonella* spp. or *Campylobacter* spp. or *Listeria monocytogenes* were not detected, while *Escherichia coli* was found in *Salsichão* and *Catalão* below 100 cfu/g (**Table IV**). Also in *Carne de Alguidar*, a homemade ready-to-eat meat product, studied in order to assess its quality and shelf-life when manufactured at the industrial scale, *L. monocytogenes* was not detected (Laranjo et al. 2016b). For ready-to-eat products, where the only established safety criteria is for *L. monocytogenes* (Reg. (EC) No. 1441/2007), these results are satisfactory. In “Alheira” made with meat from Alentejano pigs, Elias et al. (2014b) did not detect *Salmonella* spp., but *E. coli* and *L. monocytogenes* were present with values between 3 and 4 log cfu/g. Dias et al. (2015) also detected *Salmonella* spp. and *L. monocytogenes* in “Paio” manufactured with starter cultures. They found that in sausages inoculated with a lactobacilli culture, *L. monocytogenes* declined sharply and *Salmonella* spp. was eliminated. In a study conducted with “Cabeça de Xara” to assess the shelf-life of this product, low populations of *L. monocytogenes* were found in control products (less than 2 log cfu/g) and even lower in products, where vinegar was added to increase the shelf-life of the product (Laranjo et al. 2014a).

The presence of some of these pathogens may be associated with contamination occurred during slaughter and meat transport practices, but also with contamination of meat processing premises and equipment. In order to evaluate the occurrence of *Salmonella* spp., *L. monocytogenes* and *E. coli* on surfaces and equipments of three processing units, Laranjo et al. 2014b revealed the absence of *Salmonella* spp. and pre-

Table IV. Microbiological hazards found in the studied traditional products (Perigos microbiológicos encontrados nos produtos tradicionais estudados).

	Salsichão	Catalão	Chouriço preto	Paio preto	Alheira
<i>Salmonella</i> spp.	negative	negative	negative	negative	negative
<i>Campylobacter</i> spp.	negative	negative	negative	negative	ND
<i>Listeria monocytogenes</i>	negative	negative	negative	negative	2-3 log cfu/g
<i>Escherichia coli</i>	<2 log cfu/g	<2 log cfu/g	ND	ND	3-4 log cfu/g

ND: Not detected

Adapted from Elias et al. (2014b), Laranjo et al. (2015; 2106a; 2017).

Table V. Evaluation of the presence of some pathogens in three meat processing units and their equipments (Avaliação da presença de alguns microrganismos patogénicos em três unidades de processamento e equipamentos).

n = 3	Mincing machine	Mixing machine	Stuffing machine	Wall of the stuffing room
<i>Salmonella</i> spp.	Negative	Negative	Negative	Negative
<i>Listeria monocytogenes</i>	1 log cfu/cm ²	Negative	Negative	Negative
<i>Escherichia coli</i>	Negative	Negative	Negative	2,1 log cfu/cm ²

Adapted from Laranjo et al. 2014b

sence of *L. monocytogenes* and *E. coli* in reduced counts (Table V).

FINAL CONSIDERATIONS

The manufacture of processed products includes the mixing of different ingredients, which vary with product and geographic origin. These ingredients are salt, garlic, cumin, wine and vinegar that contain active principles, which contribute to control the growth of some undesirable microorganisms, even if not eliminating them completely, together with phenomena that occur along the ripening.

Data presented do not seem very worrying, since few products showed to be chemically and/or microbiologically contaminated and the majority in a reduced degree of contamination. Despite this, and observing the data presented by Xavier et al. (2014), it is always necessary to keep these hazards under control, neither neglecting the quality of raw materials nor the hygiene rules in the manufacture of meat products or the consumer information. In addition, new manufacturing processes can be developed, such as the inclusion of new ingredients and the application of innovative technologies that contribute to increase the safety of these products, gaining the confidence of consumers.

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