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## FOOD SCIENCE & TECHNOLOGY | RESEARCH ARTICLE

# Contributing data for risk assessment of traditional fermented sausages: “Salpicão de Vinhais” and “Chouriça de Vinhais”

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**Abstract:** “Salpicão de Vinhais” and “Chouriça de Vinhais” are traditional dry-fermented smoked meat sausages produced in Vinhais, a small region of Trás-os-Montes, Portugal. The scientific knowledge of this sausage variety is limited. Seventy-seven samples of “Salpicão” and “Chouriça de Vinhais” were purchased from producers, local markets and retail stores. Their microbiological and physical chemical characteristics were analysed. The same analyses were performed on the raw materials and ingredients and products during the production processes. Regarding the pathogenic flora, *Staphylococcus aureus*, spores of sulphite reducing clostridia, *Escherichia coli* 0157:H7, *Yersinia* spp. and *Salmonella* spp. were not detected in any of the samples analysed; *Listeria monocytogenes* was detected in 14.3% of the samples. The manufacturing process, namely fermentation, ripening/drying and smoking reduced the numbers of pathogen and hygiene indicator micro-organisms.

**Subjects:** Bioscience; Environment & Agriculture; Food Science & Technology

**Keywords:** “Salpicão de Vinhais” and “Chouriça de Vinhais”; microbial and chemical characterisation; *L. monocytogenes*

### 1. Introduction

“Salpicão de Vinhais” and “Chouriça de Vinhais” are traditional, dry-fermented meat sausages produced in Vinhais, a small region of Trás-os-Montes, a mountainous region in the north-east of Portugal. The essential ingredient is raw pork meat from “Bísaro”, an autochthonous Portuguese pig

### ABOUT THE AUTHOR

Paula Teixeira is integrated in Biotechnology Center and Fine Chemistry, CBQF, and leads the Food safety Research Group. This author works with global Food Safety aspects giving special attention to traditional fermented Portuguese foods. Hazards of particular interest such as *Listeria monocytogenes*—in particular but also *Campylobacter* spp. and *Staphylococcus aureus* are part of her research.

### PUBLIC INTEREST STATEMENT

“Salpicão” and “Chouriça” de Vinhais are traditional dry-fermented smoked meat sausages produced in the North of Portugal. Several samples, from different producers and retail establishments, were tested concerning global food safety aspects. *Listeria monocytogenes*, a bacterium responsible for listeriosis, a serious infection usually caused by eating food contaminated with this bacterium and considered an important public health problem worldwide, was present in the final products. The quality of raw materials and compliance with the hygiene rules at all stages of the manufacturing process are crucial to maintain the safety and the subsequent credibility of traditional Portuguese food.

breed. These sausages are registered as Protected Geographical Indication. The scientific knowledge about this sausage variety and the existing information in the scientific literature is limited (Ferreira et al., 2007, 2009). At least two slaughterhouses and three transformation units, identified by official control agencies, produce certified “Salpicão de Vinhais” and “Chouriça de Vinhais”, besides the home-made products, representing 5.9% of the national traditional sausage production. Traditional dry sausage fermentation relies on natural contamination of raw materials by microflora. Each processing facility has a specific house flora, composed of useful micro-organisms for the fermentation and flavour of sausage, as well as of spoilage and pathogenic flora (Benito, Aranda, Perez-Nevado, Ruiz-Moyano, & Córdoba, 2007; Chevallier et al., 2006). During the manufacture of dry-fermented sausages physicochemical modifications occur, especially dehydration, fermentation of carbohydrates, acidification, development of a typical colour, lipolysis and oxidation of lipids, and proteolysis due to the activity of the different microbial groups (Comi et al., 2005; Di Cagno et al., 2008; Foulquié Moreno, Sarantinopoulos, Tsakalidou, & De Vuyst, 2006; Garciafontan, Lorenzo, Parada, Franco, & Carballo, 2007). Outbreaks of foodborne illness associated with these products have occurred (Lindqvist & Lindblad, 2009; Moore, 2004; Quinto et al., 2014). Different surveys have revealed the presence of *Listeria monocytogenes*, *E. coli*, *Clostridium* spp. and coagulase-positive staphylococci not only in the final products, but also in different production environments. This can be either due to frequently contaminated raw materials, where there is a probability that some of the pathogenic organisms could cross the antimicrobial barriers imposed during processing, or to cross-contamination at any stage, including at retail.

The present work aimed to study the microbiological and chemical characterisation of “Salpicão de Vinhais” and “Chouriça de Vinhais” and to determine the relevant food safety hazards in these dry-fermented meat products.

## 2. Materials and methods

### 2.1. Origin and sampling

Samples from raw materials, products during processing and from the final products, were analysed. Raw materials (pork meat, garlic, laurel, paprika, salt and salted pork casings) and samples at different stages during processing were collected from two different processors, A and B. At least two processing batches were analysed. Samples were taken immediately and after 48 h of seasoning at 4°C, after stuffing into casings before smoking; and weekly, during the smoking process, until the end of production. A total of 48 samples of “Salpicão” and 40 samples of “Chouriça”, from both producers A and B, were collected for analysis (five or four weeks of processing for “Salpicão” or “Chouriça”, respectively × 2 duplicates per batch × 2 batches × 2 producers). Final products of “Salpicão” ( $n = 14$ ) and “Chouriça” ( $n = 13$ ) were purchased from four different local producers, at their plant. Seven samples of each product were purchased in a local market, in Vinhais. Product obtained in the last week of processing were considered as final products, in a total of 16. In addition, samples of “Salpicão” ( $n = 10$ ) and “Chouriça”, ( $n = 10$ ), from different producers, were randomly collected at retail stores. For each parameter to be evaluated, unless otherwise stated, two independent analyses were performed using randomly selected pieces.

Samples were transported to the laboratory under refrigeration (approximately 0°C, in melting ice) and were analysed within 24 h.

### 2.2. Microbiological analyses

Samples of 25 g were added to 225 mL of sterile buffered peptone water (Merck, Darmstadt, Germany), and homogenised in a stomacher for 2 min. Appropriate decimal dilutions were prepared in sterile Ringer’s solution (LabM, Bury, UK) for microbial enumeration. Aerobic mesophilic, *Enterobacteriaceae* and *Enterococcaceae*/Lactic Acid Bacteria were enumerated on Plate Count Agar (LabM), on Violet Red Bile with Lactose (VRBL, Biokar Diagnostics), and onto MRS and M17 agar (LABM), respectively, incubated at 30°C for 72 h except for VRBGA plates that were incubated at 37°C for 24 h, according to the Portuguese Standard NP4137 (Anonymous, 1991). Typical *Enterobacteriaceae*

**Table 1. Microbiological characterisation of raw materials**

| Control points | Facility | Aerobic plate count (log CFU/g) | Enterobacteriaceae (log CFU/g) | Staphylococci coagulase positive (log CFU/g) | L. monocytogenes           |         | Salmonella spp. VIDAS * (in 25 g) |
|----------------|----------|---------------------------------|--------------------------------|--|----------------------------|---------|-----------------------------------|
|                |          |                                 |                                |  | VIDAS * (presence in 25 g) | (MPN/g) |                                   |
| Pork meat      | A        | 2.8 ± 0.0                       | 2.7                            | <1   | (-)/(-)                    | <20/100 | (-)/(-)                           |
| Paprika        | A        | 4.1                             | 4.6                            | <1   | (-)/(-)                    | <20/100 | (-)/(-)                           |
|                | B        | 5.1                             | <1                             | <1   | (-)/(-)                    | <20/100 | (-)/(-)                           |
| Garlic         | A        | 2.6                             | 2.1                            | <1   | (-)/(-)                    | <20/100 | (-)/(-)                           |
|                | B        | 1.9                             | <1                             | <1   | (-)/(-)                    | <20/100 | (-)/(-)                           |
| Laurel         | A        | 2.9                             | <1                             | <1   | (-)/(-)                    | <20/100 | (-)/(-)                           |
|                | B        | 1.5                             | <1                             | <1   | (-)/(-)                    | <20/100 | (-)/(-)                           |
| Salt           | A        | <1                              | <1                             | <1   | (-)/(-)                    | <20/100 | (-)/(-)                           |
| Casings        | A        | >7.5                            | 4.1                            | <1   | (-)/(-)                    | <20/100 | (-)/(-)                           |
|                | B        | >7.5                            | 3.8                            | <1   | (-)/(-)                    | <20/100 | (-)/(-)                           |

\*Tests performed in duplicate.

**Table 2. Microbiological characterisation of “Salpicão”**

|  | Producer | Lot | Seasoning  | t (weeks)  |            |            |            |            |            |   |
|--|----------|-----|------------|------------|------------|------------|------------|------------|------------|---|
|  |          |     |            | 0          | 1          | 2          | 3          | 4          | 5          |   |
| Aerobic mesophilic plate count (log CFU/g)   | A        | 1   | 5.2 ± 0.2  | 6.6 ± 0.4  | 6.6 ± 0.0  | 7.0 ± 0.1  | 7.4 ± 0.16 | 7.4 ± 0.2  | >7.5 ± 0.0 |   |
|  |          | 2   | 5.6 ± 0.2  | >7.5 ± 0.0 | >7.5 ± 0.0 | >7.5 ± 0.0 | >7.5 ± 0.0 | >7.5 ± 0.0 | >7.5 ± 0.0 |   |
|  | B        | 1   | n.a.       | >7.5 ± 0.0 | >7.5 ± 0.0 | >7.5 ± 0.0 | >7.5 ± 0.0 | >7.5 ± 0.0 | >7.5 ± 0.0 | - |
|  |          | 2   | 5.9 ± 0.1  | 5.4 ± 0.3  | 7.3 ± 0.3  | >7.5 ± 0.0 | >7.5 ± 0.0 | >7.5 ± 0.0 | >7.5 ± 0.0 | - |
| Enterobacteriaceae (log CFU/g)               | A        | 1   | 4.3 ± 0.2  | 4.7 ± 0.1  | 4.2 ± 0.1  | 5.1 ± 0.1  | 4.2 ± 1.1  | 4.4 ± 0.3  | 3.5 ± 0.3  |   |
|  |          | 2   | 4.7 ± 0.3  | 5.1        | 3.8 ± 3.0  | 4.8 ± 0.9  | 2.4 ± 0.0  | 3.1 ± 0.5  | 3.1 ± 1.0  |   |
|  | B        | 1   | n.a.       | 3.1 ± 1.2  | 1.0 ± 0.0  | <1.0 ± 0.0 | 1.4 ± 0.6  | 3.3 ± 3.3  | -          |   |
|  |          | 2   | 3.7 ± 0.2  | <1.2       | 2.5 ± 2.1  | 1.2        | <1.0 ± 0.0 | 1.0 ± 0.2  | <1.0 ± 0.0 |   |
| Staphylococci coagulase positive (log CFU/g) | A        | 1   | 2.0 ± 1.0  | <1.0 ± 0   | 1.9 ± 0.2  | <1.0 ± 0.0 | <1.0 ± 0.0 | <2.2 ± 0.0 | <1.0 ± 0.0 |   |
|  |          | 2   | <1.0 ± 0.0 | 1.6 ± 0.8  | <1.0 ± 0   | <1.0 ± 0.0 | <1.0 ± 0.0 | <1.0 ± 0.0 | <1.0 ± 0.0 |   |
|  | B        | 1   | n.a.       | 1.4 ± 0.2  | <1.0 ± 0.0 | <1.0 ± 0.0 | <1.0 ± 0.0 | <1.0 ± 0.0 | <1.0 ± 0.0 | - |
|  |          | 2   | <1.0 ± 0.0 | <1.0 ± 0.0 | 1.6 ± 0.8  | <1.0 ± 0.0 | <1.0 ± 0.0 | <1.0 ± 0.0 | <1.0 ± 0.0 | - |
| L. monocytogenes VIDAS (presence per 25 g)   | A        | 1   | n.a.       | (-)/(-)    | (-)/(+)    | (-)/(-)    | (-)/(+)    | (-)/(-)    | -          |   |
|  |          | 2   | n.a.       | (-)/(-)    | (-)/(-)    | (-)/(-)    | (-)/(-)    | (-)/(-)    | (-)/(-)    |   |
|  | B        | 1   | n.a.       | (+)/(+)    | (-)/(+)    | (-)/(-)    | n.a.       | (-)/(-)    | -          |   |
|  |          | 2   | n.a.       | (+)/(+)    | (-)/(+)    | (+)/(+)    | (-)/(-)    | (-)/(-)    | -          |   |
| L. monocytogenes spp. (MPN/g)                | A        | 1   | n.a.       | <0.2 ± 0.0 | <0.2 ± 0.0 | <0.2 ± 0.0 | <0.2 ± 0.0 | <0.2 ± 0.0 | <0.2 ± 0.0 |   |
|  |          | 2   | n.a.       | 0.3 ± 0.2  | 0.3 ± 0.1  | 0.2 ± 0.0  | <0.2 ± 0.0 | 0.3 ± 0.1  | <0.2 ± 0.0 |   |
|  | B        | 1   | n.a.       | <0.2 ± 0.0 | <0.2 ± 0.0 | <0.2 ± 0.0 | 0.7 ± 0.7  | <0.2 ± 0.0 | -          |   |
|  |          | 2   | n.a.       | 0.5 ± 0.4  | 1.0 ± 0.9  | n.a.       | 1.9 ± 1.9  | 2.0 ± 0.4  | 1.7 ± 2.2  |   |

Notes: TVC: total viable counts; All tests performed in duplicate; n.a.: not available; A, B: producers.

colonies were confirmed by oxidase and fermentation test in glucose agar. Yeasts and moulds were enumerated on Rose Bengal agar supplemented with 0.1 g/L of chloramphenicol (Oxoid, Hampshire, UK), incubated at 25°C for 5 days; *Escherichia coli* on TBX (BioRad, Hercules, CA, USA), incubated at 44°C for 24 h and coagulase-positive staphylococci on Baird-Parker RPF agar (bioMérieux, Marcy l’Etoile, France), incubated at 37°C for 48 h, according to NF V08 057-1 (Anonymous, 2004).

**Table 3. Microbiological characterisation of “Chouriça”**

|   | Producer | Lot | Seasoning  | t (weeks)  |            |            |            |            |
|---|----------|-----|------------|------------|------------|------------|------------|------------|
|   |          |     |            | 0          | 1          | 2          | 3          | 4          |
| Aerobic mesophilic plate count (log CFU/g)        | A        | 1   | 5.8 ± 0.4  | 6.7 ± 0.1  | 7.0 ± 0.3  | 6.8 ± 0.4  | 6.08       | -          |
|   |          | 2   | 5.1 ± 0.8  | 7.4 ± 0.1  | n.a.       | >7.5 ± 0.0 | >7.5 ± 0.0 | >7.5 ± 0.0 |
|   | B        | 1   | 6.4 ± 1.0  | 6.7 ± 0.3  | >7.5 ± 0.0 | >7.5 ± 0.0 | >7.5 ± 0.0 | -          |
|   |          | 2   | 5.6 ± 0.4  | 5.1 ± 0.0  | >7.5 ± 0.0 | >7.5 ± 0.0 | >7.5 ± 0.0 | -          |
| <i>Enterobacteriaceae</i> (log CFU/g)             | A        | 1   | 4.3 ± 0.2  | 3.5 ± 0.2  | 4.6 ± 0.0  | 4.3 ± 0.1  | 4.18       | -          |
|   |          | 2   | 4.9 ± 0.1  | 4.7 ± 0.8  | 5.5 ± 0.4  | 4.9 ± 0.3  | 5.0 ± 0.5  | 4.9 ± 0.3  |
|   | B        | 1   | 4.3 ± 0.2  | 3.6 ± 0.0  | 2.4 ± 1.3  | 1.6 ± 0.2  | 1.3 ± 0.5  | -          |
|   |          | 2   | 3.8 ± 0.2  | 2.9 ± 0.8  | 1.3 ± 0.0  | <1.0 ± 0.0 | <1.0 ± 0.0 | -          |
| Staphylococci coagulase positive (log CFU/g)      | A        | 1   | <1.0 ± 0.0 | <1.0 ± 0.0 | 1.7 ± 0.3  | <1.0 ± 0.0 | <1.00      | -          |
|   |          | 2   | <1.0 ± 0.0 | <1.0 ± 0.0 | <1.0 ± 0.0 | <1.0 ± 0.0 | <1.0 ± 0.0 | <1.0 ± 0.0 |
|   | B        | 1   | <1.0 ± 0.0 | 2.0 ± 0.2  | <1.0 ± 0.0 | <1.0 ± 0.0 | 1.6 ± 0.8  | -          |
|   |          | 2   | <1.0 ± 0.0 | <1.0 ± 0.0 | <1.0 ± 0.0 | <1.0 ± 0.0 | <1.0 ± 0.0 | -          |
| <i>L. monocytogenes</i> VIDAS (presence per 25 g) | A        | 1   | n.a.       | (+)/(+)    | (+)/(+)    | (-)/(-)    | (+)/(+)    | -          |
|   |          | 2   | n.a.       | (-)/(-)    | (-)/(-)    | (-)/(-)    | (-)/(-)    | (-)/(-)    |
|   | B        | 1   | n.a.       | (+)/(+)    | (+)/(+)    | (+)/(+)    | (+)/(+)    | -          |
|   |          | 2   | n.a.       | (+)/(+)    | (+)/(+)    | (-)/(-)    | (+)/(+)    | -          |
| <i>L. monocytogenes</i> (MPN/g)                   | A        | 1   | n.a.       | <0.2 ± 0.0 | <0.2 ± 0.0 | 0.2 ± 0.0  | <0.0 ± 0.0 | -          |
|   |          | 2   | n.a.       | 3.0 ± 4.0  | n.a.       | 0.3 ± 0.1  | 0.2 ± 0.0  | 0.2 ± 0.0  |
|   | B        | 1   | n.a.       | n.a.       | 13 ± 13    | 3.0 ± 0.4  | 18 ± 9.2   | -          |
|   |          | 2   | n.a.       | 1.1 ± 0.0  | 29 ± 0.2   | n.a.       | 63 ± 41    | -          |

Sulphite-reducing *Clostridium* spores were enumerated according to the Portuguese Standard NP 2262 (Anonymous, 1986) in differential reinforced *Clostridium* medium; vegetatives cells were inactivated at 80°C during 10 min, formation of characteristic black colonies were further enumerated. The enumeration of *Listeria* spp. was performed by direct plating on PALCAM agar medium (Merck) and by the most probable number (MPN) technique using as culture media the ones referred in the ISO 11290-1 (Anonymous, 1996a), that are Demi-Fraser broth (Merck), Fraser broth (Merck) and PALCAM medium. Positive results were confirmed according to the International Standard ISO 11290-2 (Anonymous, 1998). Detection of *L. monocytogenes* was performed using the VIDAS method (Anonymous, 1996b), an enzyme-linked fluorescent immunoassay performed in the automated VIDAS instrument, using antibody specific for *L. monocytogenes* and also by direct enumeration according to the ISO 11290-2 (Anonymous, 1998). *Salmonella* spp. was detected by the VIDAS method; positive results were confirmed according to the standard techniques (Anonymous, 2002). Detection of presumptive pathogenic *Y. enterocolitica* was performed as described in ISO 10273 (Anonymous, 2003), following three successive stages: (1) enrichment in peptone, sorbitol and bile salts (PSB) broth and in irgasan™, ticarcillin and potassium chlorate (ITC) broth (Sigma-Aldrich, Germany); (2) surface plating on solid selective culture media, namely agar with cefsulodin, irgasan™ and novobiocin (CIN) (Sigma-Aldrich, Munich, Germany) and *Salmonella/Shigella* agar, with sodium desoxycholate and calcium chloride (Sigma-Aldrich). Presumptive tests such as urea-indole, Kligler and oxidase were carried out on suspected characteristic colonies, being small, smooth with a red centre and translucent rim, very finely granular when examined with obliquely transmitted light.

### 2.3. Physicochemical analyses

The pH was determined directly with a Crison MicroPH 2002 pH-meter (Crison Barcelona, Spain) equipped with an InLab 427 puncture electrode (Mettler Toledo, Columbus, OH, USA). Chloride and moisture contents were determined following the ISO Standards 1841-2 (Anonymous, 1996b) and

1442 (Anonymous, 1997), respectively. The water activity was measured with a Hygropalm AW1 (Rotronic Instrument Corporation, USA). Temperature inside one “Salpicão”, one “Chouriça” and room temperature were recorded in two different producers, A and B, using a temperature datalogger HANNAH, Instruments Model HI98804 (USA). Temperature during transportation and storage was also recorded inside one “Salpicão”, one “Chouriça” from the plant to retail store, from producer A. D-Lactic acid/L-Lactic acid in the samples of raw meat, samples of semiprocessed product and final product were determined enzymatically using D-lactate and L-lactate dehydrogenase kits (Catalog No. 1112821035, Boehringer Mannheim Roche, Germany).

### 3. Results and discussion

#### 3.1. Microbiological analyses

Regarding the raw materials used by producers A and B, results are summarised in Table 1. Pork meat and casings, as expected, showed *Enterobacteriaceae* levels between 2.7 and 4.6 log CFU/g. Total viable counts were satisfactory (<4.0 log CFU/g) for fresh meat while *Enterobacteriaceae* levels were acceptable (Anonymous, 2014). Facility A presented high counts of *Enterobacteriaceae* in paprika. Spices may carry indigenous microflora that contributes significantly to the microbial load of the sausage batter, though fresh garlic may exhibit some antimicrobial effect (Hew, Hajmeer, Farver, Glover, & Cliver, 2006). However, Kamdem, Patrignani, and Elisabetta Guerzoni (2007) showed that spices also might have an important role in the control of *L. monocytogenes* in Italian sausages. Previous work by Ferreira et al. (2009) with “Chouriça” and “Salpicão” demonstrated that the microbiological safety of these products cannot be assured if highly contaminated raw materials are used, even after smoking. The microbiological characterisation of “Salpicão” and “Chouriça” during the smoking process are summarised in Tables 2 and 3, respectively. According to the Commission Regulation (EC) No 2073/2005 (EC, 2007) and the Food Safety Authority of Ireland Guidelines (Anonymous, 2014), “Chouriça” from producer A would be classified as ‘Unsatisfactory’ as *Enterobacteriaceae* counts were higher than log 4 CFU/g. In fact, these organisms were present at high levels in all processing stages. *Staphylococcus aureus* were present in samples from both producers A and B at levels not considered hazardous (<4 log CFU/g) but still unsatisfactory (>2 log CFU/g). *L. monocytogenes* was detected in both products, from both producers; however, not in every batch and this organism seems to be reduced by the smoking process. The detection of this foodborne pathogen during the early stages of sausage fermentations, has already been discussed (Gounadaki, Skandamis, Drosinos, & Nychas, 2008; Meloni et al., 2014; Talon et al., 2007) however, according to European Food Safety Authority (EFSA); European Centre for Disease Prevention and Control (ECDC) (2014), fermented sausages contaminated with *L. monocytogenes* have rarely been implicated in critical listeriosis outbreaks. Sulphite-reducing *Clostridium* spores were not found in any sample, from both producers. The high numbers of aerobic plate counts in every sample are due, significantly, to the fermentation process and LAB development. *Enterobacteriaceae* levels were higher in producer A reaching during the smoking stages values of 5.1 log CFU/g. Within the final products ( $n = 57$ ), coagulase-positive staphylococci, spores of sulphite-reducing clostridia, *E. coli* O157:H7 and *Salmonella* spp. were not detected in any of the samples. *L. monocytogenes* was considered the pathogen of concern, being present in 15.8% of products, evenly distributed between samples of “Salpicão” and “Chouriça”. Concerning the samples collected from the retail stores ( $n = 20$ ), the presence of *L. monocytogenes*, *Yersinia* spp., *Salmonella* spp., *E. coli* O157 or *C. perfringens* was analysed. Only *L. monocytogenes* was present in 10% of the tested samples. Previously, in these products, Ferreira et al. (2007) reported *E. coli* in concentrations >100 CFU/g and coagulase-positive staphylococci in concentration <104 CFU/g. *Y. enterocolitica* has already been detected in Turkish dry-fermented sausage Sucuk (Asplund, Nurmi, Hirn, Hirvi, & Hill, 1993; Ceylan & Fung, 2000). *Salmonella* spp. and verocytotoxigenic *E. coli* were detected in fresh pork sausages (Escartin, Castillo, Hinojosa-Puga, & Saldaña-Lozano, 1999; Villani, Russo, Blaiotta, Moschetti, & Ercolini, 2005, respectively). Siriken, Pamuk, Özakın, Gedikoglu, and Eyigör (2006) detected *Salmonella* spp. and *Listeria* spp. in 7 and 9% of the samples of Turkish sausage soudjouk, while no *E. coli* O157:H7 was detected. In an Italian national survey of cacciatore salami, *L. monocytogenes* was recovered in 22.7% of the 1020 samples tested (Gianfranceschi, D’Ottavio, Gattuso, Bella, A., & Aurelia, 2009). Prevalence of *L. monocytogenes* in French dry-fermented sausages at the end of production process was 10%, with

contamination levels below 100 CFU/g (Thévenot, Delignette-Muller, Christieans, & Vernozy-Rozand, 2005). Cabedo, Picart, Barrot, and Teixido Canelles (2008) reported a prevalence of *L. monocytogenes* in several RTE food samples in Catalonia, Spain, from 1.3 to 20%. *L. monocytogenes* was not detected by Vidas or direct count techniques, only by the most probable number technique, in 90% of the samples (considering each replicate as a result, as these data will be fitted to a distribution later), though higher concentrations were detected, as previously described in this work. This is a similar result to the study conducted by Ferreira et al. (2009), where *L. monocytogenes* was not detected in 96% of the samples.

### 3.2. Chemical analysis

During the smoking process, there was an increase in both isomers D/L lactic acid, in both “Salpicão” and “Chouriça”, consistent with the previously stated growth of LAB. Genera of LAB such as *Streptococcus*, *Lactococcus*, *Enterococcus* and *Carnobacterium* produce over 90% of the L(+)-isomer as an end product of sugar fermentation. *Leuconostoc* spp. and *L. delbrueckii* (all subspecies) on the other hand produce D(-)-lactic acid (Holzapfel, Franz, Ludwig, Back, & Dicks, 2005). The nature of the lactic isomer is of concern, since high levels of the D(-)-lactic acid isomer are not hydrolysed by lactate dehydrogenase (LDH) in humans and are, thus, capable of causing acidosis (Holzapfel et al., 2005). WHO recommendations indicate a maximum daily intake of 100 mg/kg body weight of this non-physiological lactic acid isomer (WHO, 1968). There are, however, no recommended limitations for the intake of the L(+)-lactic acid isomer (Holzapfel et al., 2005). The statistical analysis indicates that the values of D-lactic acid are significantly lower than the values of L-lactic acid ( $p < 0.05$ ). High D-lactic values may be a health problem for specific patients. The values for the D- lactic acid isomer are not statistically different between the products “Salpicão” and “Chouriça” and the values for the L-lactic acid isomer are not statistically different between the products. During the smoking process, along with the growth of LAB and other microflora, the organic acid production, changes in pH, moisture content and water activity ( $a_w$ ) occur, since a drying process also occurs with the concomitant concentration effect on sodium chloride content (data not shown). A slight initial decrease was observed in pH, but the level was never below 5.0 (data not shown). The microbial stability of dry sausages is determined by the combination of different factors referred to as the “hurdleconcept” (Arnau, Serra, Comaposada, Gou, & Garriga, 2007; Thomas, Anjaneyulu, & Kondaiah, 2008; Työppönen, Petäjä, & Mattila-Sandholm, 2003).

Raw meat in the batter is kept refrigerated while it absorbs the salt; the salt decreases the initial  $a_w$  inhibiting, or at least delaying the growth of many bacteria, though it is favourable to the growth of halotolerant staphylococci.

During the fermentation, which occurs during the smoking process, high levels of LAB produce considerable amounts of lactic acid, lowering, although only slightly, the pH value (5.0) of dry sausages. Statistical comparison by two way analysis of variance indicates that no statistically significant differences were found between results from “Salpicão” and “Chouriça”, neither between producers A and B ( $p \geq 0.05$ ), for the evolution of pH, moisture content, NaCl and  $a_w$  during the smoking process. In both final products, pH varied between 5.25 and 5.50. Ferreira et al. (2009) reported pH values ranging from 5.3 to 5.4, for these types of sausages. More than 50% of samples presented total lactic acid content in the range 0.45–0.60%w/w; similar to those found in pork sausages (0.46–0.23% w/w; Capita, Llorente-Marigomez, Prieto, & Alonso-Calleja, 2006), and “chorizo de cebolla” (0.36–0.90% w/w; Castaño, García Fontán, Fresno, Tornadijo, & Carballo, 2002). Over 40% of samples presented  $a_w$  values higher than 0.92 (data not shown). According to Työppönen et al. (2003), products classified as dry-fermented sausages should present  $a_w$  values below  $a_w$  0.90. The EC Regulation 2073/2005 (EC, 2007) classifies as unable to support growth of *L. monocytogenes*, food products with  $a_w$  equal or inferior to 0.92. Thus it would be expected that 40% of samples could support the growth of *L. monocytogenes*.

#### 4. Conclusions

“Salpicão” and “Chouriça” use very similar raw materials; in both facilities, pathogens of concern were not detected. In the final products however, *L. monocytogenes* was detected highlighting the risk of these products and the need to apply hurdles to control the contamination of *L. monocytogenes* in meat processing plants. In addition, good manufacturing practices, correct sampling schemes, adequate cleaning and disinfection procedures and HACCP principles have to be applied.

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#### Competing interests

The authors declare no competing interest.

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#### References

- Anonymous. (1997). *ISO meat and meat products—Determination of moisture content*. ISO 1442.
- Anonymous. (1991). *General guidance for the enumeration of enterobacteriaceae without resuscitation*. MPN technique and colony count technique, Portuguese Standard NP 4137.
- Anonymous. (2014). *Guidelines for the interpretation of results of microbiological testing of ready to eat foods placed on the market* (Revision 1). Food Safety Authority of Ireland.
- Anonymous. (2002). *Microbiology of food and animal feeding stuffs—Horizontal method for the detection of Salmonella spp.* International Standard ISO 6579.
- Anonymous. (2003). *Microbiology of food and animal feeding stuffs—Horizontal method for the detection of presumptive pathogenic Yersinia enterocolitica* ISO 10273:2003.
- Anonymous. (2004). *Microbiologie des aliments—Méthode de routine pour le dénombrement des staphylocoques à coagulase positive par comptage des colonies à 37°C—Partie 1: technique avec confirmation des colonies*. NF V08-057-1.
- Anonymous. (1986). *IPQ food microbiology. General rules for identifying reducing clostridium sulphite spores*. NP 2262.
- Anonymous. (1996a). *Microbiology of food and animal feeding stuffs—Horizontal method for the detection and enumeration of Listeria monocytogenes—Part 1: Detection method* ISO 11290-1:1996.
- Anonymous. (1996b). *ISO meat and meat products—Determination of chloride content—Part 2: Potentiometric method*. ISO1841-2.
- Anonymous. (1998). *Microbiology of food and animal feeding stuffs—Horizontal method for the detection and enumeration of Listeria monocytogenes—Part 2: Enumeration method*. ISO 11290-2/Amd 1:2004.
- Arnau, J., Serra, X., Comaposada, J., Gou, P., & Garriga, M. (2007). Technologies to shorten the drying period of dry-cured meat products. *Meat Science*, 77, 81–89. <http://dx.doi.org/10.1016/j.meatsci.2007.03.015>
- Asplund, K., Nurmi, E., Hirn, J., Hirvi, T., & Hill, P. (1993). Survival of *Yersinia enterocolitica* in fermented sausages manufactured with different levels of nitrite and different starter cultures. *Journal of Food Protection*, 56, 710–712.
- Benito, M. J., Aranda, M. E., Perez-Navado, F., Ruiz-Moyano, S., & Córdoba, M. G. (2007). Characterization and selection of autochthonous lactic acid bacteria isolated from traditional iberian dry-fermented salchichón and chorizo sausages. *Journal of Food Safety*, 72, 198–201.
- Cabedo, L., Picart, L., Barrot, I., & Teixido Canelles, A. (2008). Research note prevalence of *Listeria monocytogenes* and *Salmonella* in ready-to-eat food in Catalonia, Spain. *Journal of Food Protection*, 71, 855–859.
- Capita, R., Llorente-Marigomez, S., Prieto, M., & Alonso-Calleja, C. (2006). Microbiological profiles, pH and titratable acidity of chorizo and salchichón (two Spanish dry-fermented sausages) manufactured with ostrich, deer, or pork meat. *Journal of Food Protection*, 69, 1183–1189.
- Castaño, A., Garcia Fontán, M. C., Fresno, J. M., Tornadijo, M. E., & Carballo, J. (2002). Survival of *Enterobacteriaceae* during processing of chorizo de cebolla, a Spanish fermented sausage. *Food Control*, 13, 107–115. [http://dx.doi.org/10.1016/S0956-7135\(01\)00089-5](http://dx.doi.org/10.1016/S0956-7135(01)00089-5)
- Ceylan, E., & Fung, D. Y. C. (2000). Destruction of *Yersinia enterocolitica* by *Lactobacillus sake* and *Pediococcus acidilactici* during low-temperature fermentation of Turkish dry sausage (sucuk). *Journal of Food Science*, 65, 876–879. <http://dx.doi.org/10.1111/jfds.2000.65.issue-5>
- Chevallier, I., Ammor, S., Laguet, A., Labayle, S., Castanet, V., Dufour, E., & Talon, R. (2006). Microbial ecology of a small-scale facility producing traditional dry sausage. *Food Control*, 17, 446–453. <http://dx.doi.org/10.1016/j.foodcont.2005.02.005>
- Comi, G., Urso, R., Iacumin, L., Rantsiou, K., Cattaneo, P., Cantoni, C., & Coccolin, L. (2005). Characterisation of naturally fermented sausages produced in the North East of Italy. *Meat Science*, 69, 381–392. <http://dx.doi.org/10.1016/j.meatsci.2004.08.007>
- Di Cagno, R., López, C., Tofalo, R., Gallo, G., De Angelis, M., Paparella, A., ... Gobbetti, M. (2008). Comparison of the compositional, microbiological, biochemical and volatile profile characteristics of three Italian PDO fermented sausages. *Meat Science*, 79, 224–235. <http://dx.doi.org/10.1016/j.meatsci.2007.09.006>
- EC. (2007). Commission Regulation (EC) No. 1441/2007 of 5 December 2007 amending regulation EC No. 2073/2005 on microbiological criteria for foodstuffs. *Official Journal of the European Union*, L 322/12.
- Escartin, E. F., Castillo, A., Hinojosa-Puga, A., & Saldaña-Lozano, J. (1999). Prevalence of *Salmonella* in chorizo and its survival under different storage temperatures. *Food Microbiology*, 16, 479–486. <http://dx.doi.org/10.1006/fmic.1999.0258>

- European Food Safety Authority (EFSA); European Centre for Disease Prevention and Control (ECDC). (2014). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2012. *EFSA Journal*, 12, 3547.
- Ferreira, V., Barbosa, J., Silva, J., Vendeiro, S., Mota, A., Silva, F., João Monteiro, M., & Hogg, T. (2007). Chemical and microbiological characterisation of “Salpicão de Vinhais” and “Chouriça de Vinhais”: Traditional dry sausages produced in the North of Portugal. *Food Microbiology*, 24, 618–623. <http://dx.doi.org/10.1016/j.fm.2006.12.007>
- Ferreira, V., Barbosa, J., Silva, J., Gibbs, P., Hogg, T., & Teixeira, P. (2009). Microbiological profile of Salpicão de Vinhais and Chouriça de Vinhais from raw materials to final products: Traditional dry sausages produced in the North of Portugal. *Innovative Food Science and Emerging Technologies*, 10, 279–283. <http://dx.doi.org/10.1016/j.ifset.2008.11.001>
- Foulquié Moreno, M. R., Sarantinopoulos, P., Tsakalidou, E., & De Vuyst, L. (2006). The role and application of enterococci in food and health. *International Journal of Food Microbiology*, 106, 1–24. <http://dx.doi.org/10.1016/j.ijfoodmicro.2005.06.026>
- Garciafontan, M., Lorenzo, J., Parada, A., Franco, I., & Carballo, J. (2007). Microbiological characteristics of “androlla”, a Spanish traditional pork sausage. *Food Microbiology*, 24, 52–58. <http://dx.doi.org/10.1016/j.fm.2006.03.007>
- Gianfranceschi, M., D’Ottavio, V., M. C., Gattuso, A., Bella, A., & Aurelia, P. (2009). Distribution of serotypes and pulsotypes of *Listeria monocytogenes* from human, food and environmental isolates (Italy 2002–2005). *Food Microbiology*, 26, 1–7.
- Gounadaki, A., Skandamis, P., Drosinos, E., & Nychas, G. J. (2008). Microbial ecology of food contact surfaces and products of small scale facilities producing traditional sausages. *Food Microbiology*, 25, 313–323. <http://dx.doi.org/10.1016/j.fm.2007.10.001>
- Hew, C. M., Hajmeer, M. N., Farver, T. B., Glover, J. M., & Cliver, D. O. (2006). Pathogen survival in chorizos: Ecological factors. *Journal of Food Protection*, 69, 1087–1095.
- Holzappel, W. H., Franz, C. M. A. P., Ludwig, W., Back, W., & Dicks, L. M. T. (2005). The genera *Pediococcus* and *Tetragenococcus*. In M. Dworkin, S. Falkow, E. Rosenberg, K.-H. Schleifer, & E. Stackebrandt (Eds.), *The prokaryotes: An evolving electronic resource for the microbiological community* (3rd ed., pp. 229–266, release 3.2). New York, NY: Springer-Verlag.
- Kamdem, S. S., Patrignani, F., & Elisabetta Guerzoni, M. (2007). Shelf-life and safety characteristics of Italian Toscana traditional fresh sausage (Salsiccia) combining two commercial ready-to-use additives and spices. *Food Control*, 18, 421–429. <http://dx.doi.org/10.1016/j.foodcont.2005.11.013>
- Lindqvist, R., & Lindblad, M. (2009). Inactivation of *Escherichia coli*, *Listeria monocytogenes* and *Yersinia enterocolitica* in fermented sausages during maturation/storage. *International Journal of Food Microbiology*, 129, 59–67. <http://dx.doi.org/10.1016/j.ijfoodmicro.2008.11.011>
- Meloni, D., Consolati, S. G., Mazza, R., Mureddu, A., Fois, F., Piras, F., & Mazzette, R. (2014). Presence and molecular characterization of the major serovars of *Listeria monocytogenes* in ten Sardinian fermented sausage processing plants. *Meat Science*, 97, 443–450. <http://dx.doi.org/10.1016/j.meatsci.2014.02.012>
- Moore, J. E. (2004). Gastrointestinal outbreaks associated with fermented meats. *Meat Science*, 67, 565–568. <http://dx.doi.org/10.1016/j.meatsci.2003.12.009>
- Quinto, E. J., Arinder, P., Axelsson, L., Heir, E., Holck, A., Lindqvist, R., ... Pin, C. (2014). Predicting the concentration of verotoxin-producing *Escherichia coli* bacteria during processing and storage of fermented raw-meat sausages. *Applied and Environmental Microbiology*, 80, 2715–2727. <http://dx.doi.org/10.1128/AEM.03791-13>
- Sırken, B., Pamuk, Ş., Özakin, C., Gedikoglu, S., & Eyigör, M. (2006). A note on the incidences of *Salmonella* spp., *Listeria* spp. and *Escherichia coli* O157:H7 serotypes in Turkish sausage (Soudjouck). *Meat Science*, 72, 177–181. <http://dx.doi.org/10.1016/j.meatsci.2005.05.025>
- Talon, R., Lebert, I., Lebert, A., Leroy, S., Garriga, M., Aymerich, T., ... Fraqueza, M. J. (2007). Traditional dry fermented sausages produced in small-scale processing units in Mediterranean countries and Slovakia. 1: Microbial ecosystems of processing environments. *Meat Science*, 77, 570–579. <http://dx.doi.org/10.1016/j.meatsci.2007.05.006>
- Thévenot, D., Delignette-Muller, M. L., Christieans, S., & Vernozy-Rozand, C. (2005). Fate of *Listeria monocytogenes* in experimentally contaminated French sausages. *International Journal of Food Microbiology*, 101, 189–200. <http://dx.doi.org/10.1016/j.ijfoodmicro.2004.11.006>
- Thomas, R., Anjaneyulu, A. S. R., & Kondaiah, N. (2008). Effect of hot-boned pork on the quality of hurdle treated pork sausages during ambient temperature (37±1°C) storage. *Food Chemistry*, 107, 804–812. <http://dx.doi.org/10.1016/j.foodchem.2007.08.079>
- Työppönen, S., Petäjä, E., & Mattila-Sandholm, T. (2003). Bioprotectives and probiotics for dry sausages. *International Journal of Food Microbiology*, 83, 233–244. [http://dx.doi.org/10.1016/S0168-1605\(02\)00379-3](http://dx.doi.org/10.1016/S0168-1605(02)00379-3)
- Villani, F., Russo, F., Blaiotta, G., Moschetti, G., & Ercolini, D. (2005). Presence and characterisation of verotoxin producing *E. coli* in fresh Italian pork sausages, and preparation and use of an antibiotic resistant strain for challenge studies. *Meat Science*, 70, 181–188. <http://dx.doi.org/10.1016/j.meatsci.2004.12.010>
- WHO. (1968). *Food additives*, 29, 144–148.



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