Chemical and microbiological characterisation of "Salpicão de Vinhais" and "Chouriça de Vinhais": Traditional dry sausages produced in the North of Portugal

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Keywords: Microbiological characterisation; Chemical properties; Traditional smoked sausages; Vinhais; "Salpicão de Vinhais"; "Chouriça de Vinhais"

Abstract

"Chouriça de Vinhais" and "Salpicão de Vinhais" are traditional smoked naturally fermented meat products produced in the North of Portugal, Trás-os-Montes. The objective of this study was the characterisation of these products, giving particular attention to their microbiological and chemical safety. Nitrite, nitrate, heavy metals and biogenic amines were within accepted limits for meat products. Globally, the need for improvements in the good manufacturing practices was demonstrated in this study as various lots were considered of unsatisfactory microbiological quality according to the guidelines published by the Food Safety Authority of Ireland.

Introduction

Since the 18th century "Salpicão de Vinhais" and "Chouriça de Vinhais", traditional sausages made of *Bísaro* (an autochthonous breed of pig of N. Portugal, Trás-os-Montes), or cross-bred Bísaro pork meat, have been part of the daily diet in the region of Vinhais, Trás-os-Montes. Both products are made from raw pork meat marinated in a mixture of salt, water, wine from the region and spices, adjusted to the desirable taste (variable according to the producer). In the production of "Chouriça" small pieces of meat and fat are used while in the production of "Salpicão" bigger pieces and only lean meats from loin are used. After 48 h at 4 °C, the mixture is stuffed into pork intestine (in the case of "Chouriça"—horseshoe-shaped sausages, ca. 30–35 cm long; in the case of "Salpicão" cylindrical formed sausages, ca. 15–20 cm long) and then smoked during 3–4 weeks. Although not being generally controlled, smoking temperatures are generally about 20–30 °C. Normally these products are consumed without further cooking.

The denomination of 'Protected Geographic Indication' (PGI), was attributed to these products by the Commission Regulation (EC) No 1265/98 of 18 June 1998.

Fermented sausages are considered safe meat products due to the reduction in a_w and pH that occurs during processing and storage, which together inhibit the development of pathogenic microflora. It has been reported by several authors that fermented dry sausages and other meat products could contain, during processing and in the final product, some of the well-known pathogenic bacteria often associated with meat products, such as *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* spp., *Staphylococcus aureus*, *Clostridium perfringens* and *Campylobacter* spp. (Moore, 2004; Santos et al., 2005; Ferreira et al., 2006, 2007; Siriken et al., 2006). In addition to microbiological hazards, the presence of heavy metals and chemical agents, that can also be a health concern for consumers, has been reported (Cruz et al., 2003; Komprda et al., 2004).

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The aim of this study was to evaluate the microbiological and chemical characterisation of ''Salpicão de Vinhais'' and ''Chouriça de Vinhais''. The guarantee of the safety of consumption of these products is necessary in order to improve their recognition, as protected names are mainly related to organoleptic and chemical characterisation.

Methods

Sampling

''Chouriça'' and ''Salpicão'' from four different producers were collected from retail establishments during the period February 2004–September 2005; with the exception of producer 4, products were analysed at least in two different periods (different batches) during the study. Samples were transported to the laboratory in portable, insulated cold-boxes and stored at 4 °C until they were analysed. For each parameter to be evaluated, unless otherwise stated, two independent analyses were performed using randomly selected pieces.

Chemical analyses

The proximate composition in terms of proteins, fat and carbohydrates was determined according to Slack (1997). Nitrite, nitrate, chloride and moisture contents were determined following the ISO Standards 2918 (Anonymous, 1975a), 3091 (Anonymous, 1975b), 1841-2 (Anonymous, 1996a) and 1442 (Anonymous, 1997), respectively. 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). Lead, cadmium, arsenic and mercury were determined by atomic absorption spectrometry. Arsenic was determined according to AOAC Methods (1997). Mercury was determined according to the European Standard EN 13806 (Anonymous, 2002a). Cadmium and lead were determined according to the European Standard EN 14082 (Anonymous, 2003). The presence of biogenic amines was evaluated in one lot of ''Chouriça'' and one lot of ''Salpicão'', both from producer A. A sample of 10 g each was weighed into an 85 ml test tube and extracted with *o*-phthaldialdehyde (OPA) and biogenic amines were determined by HPLC using a method based on that described by Komprda et al. (2004).

Microbiological analyses

Twenty-five gram samples 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. Lactic acid bacteria counts were determined on de Man, Rogosa Sharpe Agar (MRS, LabM) and on M17 (Lab M), incubated at 30 °C for 72 h; *Enterococcaceae* on Bile Esculin Azide Agar (Biokar Diagnostics, Beauvais, France), incubated at 30 °C for 72 h; *Micrococcaceae* on Mannitol Salt Agar (Biokar Diagnostics), incubated at 37 °C for 48 h; yeasts and moulds on Rose-bengal Agar supplemented with 0.1 g/L of chloramphenicol (Oxoid, Hampshire, UK), incubated at 25 °C for 5 days; *E. coli* on TBX (BioRad, CA), incubated at 44 °C for 24 h; coagulase-positive staphylococci on Baird-Parker RPF-agar (bioMérieux, Marcy l'Etoile, France), incubated at 37 °C for 48 h; *Enterobacteriaceae* according to ISO 21528-2 (Anonymous, 2000); sulphite reducing *Clostridium* spores according to the Portuguese Standard NP 2262 (Anonymous, 1986); enumeration of *C. perfringens* was performed according to the European Standard EN 13401 (Anonymous, 1999).

Detection of *E. coli* O157 was performed by Immuno-Magnetic Separation using Dynabeads® (Dynal A.S., Oslo, Norway) coated with absorbed and affinity-purified anti-*E. coli* O157 antibodies according to the *International Standard*, ISO 16654 (Anonymous, 2001a).

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 by direct enumeration according to the International Standard ISO 11290-2 (Anonymous, 1998). *Salmonella* spp. were also detected by the VIDAS method (Anonymous, 1994). Positive results were confirmed using as enrichment broths Rappaport-Vassiliadis and Muller-Kauffmann Tetrathionate according to the standard techniques described in ISO 6579 (Anonymous, 2002b).

Results and discussion

The physicochemical characteristics of the samples analysed are shown in Table 1. The high levels of NaCl (decrease of water activity) and low pH (5.9–5.0) when judged ready for marketing, are characteristics that display an unique organoleptic and sensory profile in the products and are also important 'hurdles' for the growth of the pathogenic bacteria, ensuring the safety of these products. The highest pH value (5.9 in a sample of ''Salpicão'') that would have permitted growth of the pathogens, however, did not coincide with a low salt concentration (6.2% salt-on-water). The moisture levels in ''Chouriça'' were lower that in ''Salpicão'', although the final pH values were similar (Table 1). There appeared to be no correlation between salt-on-water levels and pH values in these products, suggesting that other factors were involved in enhancing or suppressing fermentation, possibly the initial levels and types of lactic acid bacteria (LAB).

LAB were the dominant microflora (Tables 3 and 4) for both products. With a few exceptions, counts on MRS and on M17 exceeded 7.0 log cfu/g. With the exception of samples from producer 2, batches C, where no growth was observed, counts on Mannitol Salt Agar ranged from 3.2 to 4.5 log cfu/g. Lactic acid production during growth of LAB

Table 1
Minimum, maximum, mean and standard deviations of physico-chemical parameters and composition of "Salpicão de Vinhais" and "Chouriça de Vinhais"

	"Salpicão"				"Chouriça"			
	Min.	Max.	Mean	SD	Min.	Max.	Mean	SD
pH	5.0	5.9	5.3	0.2	5.1	5.6	5.4	0.1
% NaCl	1.5	4.3	2.4	0.8	1.3	2.9	2.3	0.5
% Moisture	30.1	57.3	41.7	8.9	13.9	44.0	29.8	9.4
% Fat	3.6	26.4	16.2	8.0	20.4	68.2	36.1	14.3
% Total protein	31.6	43.5	35.3	4.0	12.3	35.9	27.2	6.7
% Carbohydrates	<0.1	16.4	3.2	5.3	<0.1	21.3	3.7	67
Energy (kcal/100 g)	176.0	374.0	299.7	70.4	329.0	497.0	412.0	68.0

Table 2
Biogenic amine content in "Chouriça de Vinhais" and "Salpicão de Vinhais" (mg/kg)

Product/lot	Histamine	Methylamine	Ethylamine	Tyramine	2-Phenyl ethylamine	Putrescine	Isoamylamine	Cadaverine
"Salpicão" 1 A	0.2	0.0	0.0	13.4	0.5	0.4	0.0	0.1
"Chouriça" 1 A	1.6	0.6	0.6	75.9	0.0	11.3	0.0	1.7

influences the sensorial characteristics of the product; decrease of pH and subsequent coagulation of the soluble proteins gives the desirable texture. LAB can also produce inhibitory substances against food-borne pathogens, e.g. bacteriocins (Montel et al., 1998).

For all "Salpicão" and "Chouriça" samples that were analysed, nitrate concentrations (data not shown; <66 mg/kg) and nitrite concentrations (data not shown; <11 mg/kg) were lower than the accepted limits for these parameters by the European Commission (1995) (Anonymous, 1995); 250 mg/kg in the case of nitrate and 100 mg/kg in the case of nitrite. It is noteworthy that, unlike many similar products, mainly those produced at industrial level, "Salpicão de Vinhais" and "Chouriça de Vinhais" are not traditionally prepared using nitrate or nitrite and thus it is expected that the levels of these compounds will be very low.

The Commission Regulation No. 466/2001 (Anonymous, 2001b) defined the maximum levels of cadmium (0.05 mg/kg) and lead (0.1 mg/kg) in meat products. In our study, these legislated maximum values were never exceeded (data not shown). One Portuguese fermented meat sample had already been reported to exceed the value legislated for lead (0.6 mg/kg) (Ferreira et al., 2006, 2007); high lead concentration in a Greek fermented meat product was also reported by the Directorate-General Health and Consumer Protection, 2004; 0.7750 mg/kg (Anonymous, 2004).

There is presently no legislation in force for arsenic and mercury in meats within EU. Levels found in all the samples analysed (data not shown) were within the range of values reported by the Directorate-General Health and Consumer Protection (2004) for meat and meat products produced in Denmark, Germany, Ireland and U.K., 0.0037–0.033 mg/kg (Anonymous, 2004).

Tyramine and putrescine were the amines with the highest concentration in the case of "Chouriça" and tyramine in the case of "Salpicão" (Table 2). Tyramine has been reported as the most abundant amine in fermented sausages (Ferreira et al., 2007). The toxic level of tyramine is 100–800 mg/kg (Silla-Santos, 1996). According to this, biogenic amines in the "Chouriça" and "Salpicão" analysed, apparently do not represent an obvious hazard. However, more products need to be analysed as a high variability in the biogenic amines content was previously reported for Portuguese sausages produced by different manufacturers (Ferreira et al., 2007).

Proteolytic and lipolytic activity, particularly by micrococci, leads to the production of desirable flavours and it has been demonstrated that these organisms play an important role in the manufacture of various traditional fermented sausages, namely in aroma development (Montel et al., 1998; Garcia-Varona et al., 2000). Yeasts and moulds were isolated from most of the products ranging from 2.38 log to 6.11 log and from less than 1 log to 5.11 log cfu/g, respectively. It is well accepted that these isolates might play an important role in the definition of the organoleptic profile of the final product (Ferreira et al., 2006, 2007).

With the exception of one lot of "Chouriça de Vinhais" from producer 1, enterococci counts were higher than 4.0 log cfu/g. Strains of this genus are frequently isolated

from fermented sausages and are described as being involved in flavour and texture development during fermentation (Ferreira et al., 2006, 2007). The presence of *Enterococcus* spp. in foods, however, is a concern, as many strains possess virulence factors and are becoming increasingly resistant to antibiotics; of particular concern is resistance to vancomycin (Klein, 2003; Hayes et al., 2004; Ferreira et al., 2006, 2007). Presently, there are no established limits for the enterococci (Arony, 1992).

Enterobacteria are natural inhabitants of the gastrointestinal tract of animals. Their presence in smoked sausages may be derived from contamination of raw materials or the utensils used for cutting up, or handling. As observed in Tables 3 and 4, counts of *Enterobacteriaceae* in the sausages were variable between producers and even between batches from the same producer. Although *Enterobacteriaceae* are considered useful indicators of post-processing contamination of heat processed foods (Anonymous, 2001c), the conditions during manufacturing, as previously described by other authors (Castaño et al., 2002), might have been insufficient to eliminate *Enterobacteriaceae*. Since temperature is not controlled during processing, variations in these parameters could possibly explain the variability observed in the results. The presence of *E. coli* in fermented meat products has been reported; this organism is a common contaminant of raw meat, but usually disappears from fermented sausages as a result of the combined effects of low pH and low a_w (Castaño et al., 2002). As observed in Tables 3 and 4, *E. coli* was only detected in concentrations higher than 100 cfu/g in a few lots, suggesting the possibility of limited growth of the organism during the process. *S. aureus* was recovered only from one lot of "Salpicão" (Lot A from producer 3; data not shown) but in a concentration lower than 10^4 cfu/g; even though *S. aureus* is known to be very tolerant to low levels of a_w it is not a "good competitor" namely with high numbers of LAB (Incze, 1998).

In all samples of "Salpicão" and "Chouriça", no *Salmonella* spp., *E. coli* O157 or *C. perfringens* were detected. *L. monocytogenes* was detected only in three "Chouriça" products (in batches B and C from producer 1 and in batch A from producer 3); only in one sample did numbers exceed 100 cfu/g. *L. monocytogenes* has been recognised as one of the most important foodborne pathogens found in fermented dry products (Thévenot et al., 2005). It is frequently found in raw materials and also during further transformation processes, in which it can be introduced; initial numbers may increase or decrease depending upon the extent of cross-contamination, personal and general hygienic measures and the process parameters (Farber and Peterkin, 1991; Uyttendaele et al., 1999).

The guidelines of the Food Safety Authority of Ireland (Anonymous, 2001c) were followed in the microbiological classification of each lot (Figs. 1 and 2). The unsatisfactory classification of samples of "Salpicão" and "Chouriça",

Table 3
Microbiological characterization of "Salpicão de Vinhais"

Producer	Lot	Counts on MRSA (log cfu/g)	Counts on M17 (log cfu/g)	Counts on MSA (log cfu/g)	Enterococci (log cfu/g)	Moulds (log cfu/g)	Yeasts (log cfu/g)	Enterobacteriaceae	SRC spores[a]	*E. coli*
1	A	5.2±0.1	5.8±0.0	4.5±0.2	4.0±0.0	2.2±0.7	4.7±0.1	<2.2±0.0	(−) 1 g (−) 1 g	<1
	B	6.9±1.0	7.0±0.2	3.2±0.2	4.1±0.1	1.9±1.3	3.4±0.1	5.8±0.1	(−) 1 g (−) 1 g	<1
	C	8.5±0.2	8.4±0.0	3.5±0.0	6.0±0.0	3.5±1.8	4.0±0.9	3.2±0.4	(+) 1 g/(−) 0.1 g (−) 1 g	2.4±0.1
2	A	7.5±0.3	6.6±1.2	3.5±0.1	5.9±0.1	2.5±0.1	2.6±0.1	2.3±0.0	(−) 1 g (−) 1 g	<1
	B	8.8±0.2	8.6±0.1	3.5±0.0	4.7±0.1 <2.2±0.0	<1.0±0.0	2.4±1.1	3.4±0.0	(−) 1 g	
	C	6.0±0.0	7.9±0.0		<1.0±0.0 <1.0±0.0	5.5±0.1 (−) 1 g	<1	<2.2±0.0	(+) 1 g/(−) 0.1 4.3±0.0 (+) 1 g/(−) 0.1	
3	A	8.4±0.1	8.4±0.0	3.8±0.1	5.5±0.1	4.2±1.2	5.6±0.0	2.6±0.1	(+) 0.1 g/(−) 0.01 g (+) 0.1 g/(−) 0.01 g	<1
	B	7.8±0.2	7.7±0.2	3.8±0.1	7.1±0.0	2.8±0.7	3.8±0.1	6.6±0.9	(−) 1 g (−) 1 g	4.6±1.0
4	A	9.0	9.0	3.7	7.2	5.1	6.1	4.0	(−) 1 g	3.1

[a]SRC spores; sulphite reducing clostridial spores, presence (+) or absence (−) in 1, 0.1 or 0.01 g sample.

Table 4
Microbiological characterization of "Chouriça de Vinhais"

Producer	Lot	Counts on MRSA (log cfu/g)	Counts on M17 (log cfu/g)	Counts on MSA (log cfu/g)	Enterococci (log cfu/g)	Moulds (log cfu/g)	Yeasts (log cfu/g)	Enterobacteriaceae	SRC spores	*E. coli*
1	A	5.9±0.4	6.38±0.3	4.5±0.5	3.3±0.2	<1.0±0.0	3.7±0.4	2.9±1.0	(−) 1 g (−) 1 g	< 1
	B	7.3±0.8	6.98±1.2	3.4±0.3	6.2±0.3	3.0±0.1	4.0±0.0	5.8±0.1	(−) 1 g (−) 1 g	< 1
	C	7.8±0.8	7.74±0.9	3.4±0.3	5.4±0.1	3.6±0.3	5.7±0.2	3.9±0.4	(−) 1 g (−) 1 g	< 1
2	A	7.6±0.1	7.53±0.1	3.8±0.6	5.0±1.3	2.9±0.3	4.8±0.5	<2.2±0.0	(+) 1 g/(−) 0.1 g (−) 1 g	< 1
	B	7.2±1.1	7.41±0.9	3.9±0.4	4.38±0.0	1.8±0.1	3.7±0.1	2.9±0.6	(−) 1 g (−) 1 g	< 1
	C	7.0±0.6	6.74±0.3		<1.0±0.0 <1.0±0.0	5.3±0.4 (−) 1 g	< 1	<1.0±0.0	3.3±0.2 (−) 1 g	
3	A	7.5±0.5	7.3±0.4	4.1±0.1	4.4±0.2	2.6±0.0	5.8±0.1	2.9±0.7	(+) 0.1 g/(−) 0.01 g (−) 1 g	< 1
	B	8.8±0.1	8.2±0.1	4.2±0.6	7.0±0.1	4.1±0.0	3.9±0.0	6.0±0.7	(−) 1 g (−) 1 g	5.2±0.2
4	A	8.4	8.5	3.8	>7.2	3.7	4.7	6.0	(+) 0.1 g/(−) 0.01 g	5.5

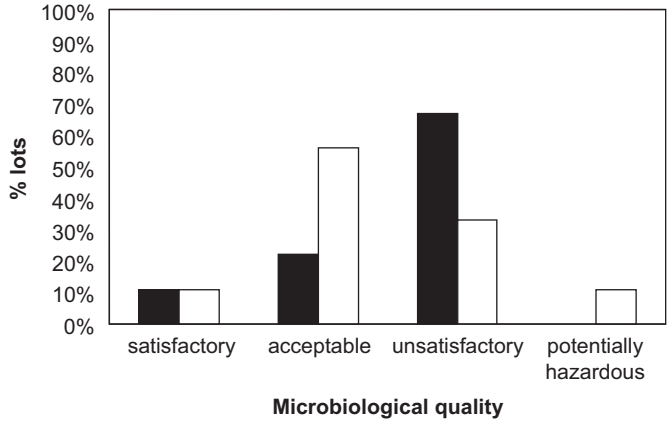

Fig. 1. Classification attributed to lots, according to the Irish Guidelines (Anonymous, 2001c), of Salpicão (■) and Chouriça (□).

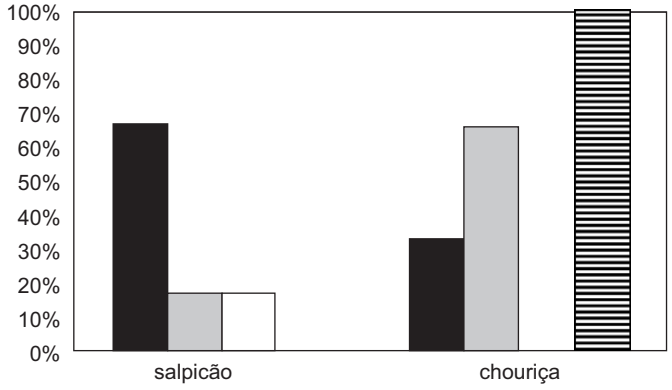

Fig. 2. Percentage of each organism or group of organisms that contributed to the classification unsatisfactory (■, *Enterobacteriaceae*; □, *Enterobacteriaceae* + *S. aureus*; ▩, *Enterobacteriaceae* + *E. coli*) or potentially hazardous (▤, *L. monocytogenes*).

resulted from excessive contamination with *Enterobacteriaceae* or these organisms combined with *E. coli* or *S. aureus* (Fig. 2); the 'potentially hazardous' sample of "Chouriça" was classified on the basis of the counts of *L. monocytogenes* exceeding 100 cfu/g (Fig. 2).

As a general conclusion, although only one lot had been considered potentially hazardous, results clearly indicated a need for improvement in the manufacturing and hygienic practices for these products. As the LAB form the majority of the microflora, it is possible that some of these organisms may be bacteriocin producers that exert some control over pathogens. Further work is also necessary to define the temperature profiles during manufacture, the progress of fermentation and pH, loss of moisture (decrease in a_W), and sources of pathogens in these products.

Acknowledgement

This work was supported by FCT/FEDER project POCTI/AGG/39587/2001.

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