Chemical and microbiological characterization of *alheira*: A typical Portuguese fermented sausage with particular reference to factors relating to food safety

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Abstract

Alheiras are traditional smoked naturally fermented meat sausages produced in the north of Portugal. They have not previously been characterized as to their chemical and microbiological status. pH and salt levels are insufficient to assure microbiological safety, there is ample opportunity for post-cooking contamination; the products require chill storage and cooking before consumption.

Heavy metals and biogenic amines were, in general, within accepted limits for meat products. Lactic acid bacteria comprised the major microflora (ca. 7–8 log cfu/g) with substantial counts of micrococci and enterococci (up to 7 log cfu/g). *Escherichia coli, Staphylococcus aureus* and *Listeria* spp. were detected in several samples.

Introduction

Fermented meat products are part of the daily diet in rural areas of Portugal and fashionable food products in urban centres whose market has been increasing in a significant way. The physicochemical and microbiological characteristics of some traditional meat products, mainly Spanish and Italian, have been studied by other research groups (e.g. Drosinos et al., 2005; Moretti et al., 2004; Santos et al., 2005). Little information, however, exists on the Portuguese products.

Fermented sausages are considered safe foods due to the reduction in a_w and pH that occurs during processing and storage and inhibits the development of pathogenic bacteria. The probability that some of the pathogenic organisms could overcome the antimicrobial hurdles imposed during processing and that they may be present in the final product has, however, been a concern for producers and those responsible for public health and has been the topic of study of several research groups. In fact, various gastrointestinal disease outbreaks associated with fermented meats were reported by Moore (2004). In addition to microbiological hazards, the presence of biogenic amines in fermented sausages has been reported (Suzzi & Gardini, 2003). The excessive consumption of these amines can be a health concern (ten Brink, Damink, Joosten, & in 't Veld, 1990).

Alheiras are traditional, smoked, naturally-fermented meat sausages, produced in the North of Portugal. The origin of *alheiras* goes back to the end of the fifteenth century and it is associated with the presence of Jewish communities in Trás-os-Montes, after they were banned from Castile in 1492. According to tradition, a type of sausage was developed which was similar in shape to those that were part of Christian cuisine, but which was stuffed with

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chicken and flour instead of pork meat and fat. In this way, the crypto-Jews escaped being identified by the inquisition because of their different eating habits. The recipe eventually became popular among Christians and nowadays, in addition to the home-made *alheiras*, more than 500 tons are produced annually by various commercial units, using pork as well as other types of meats (duck, turkey, partridge and veal), representing an important economic resource for the region (http://www.idrha.min-agricultura.pt/produtos_tradicionais/estatisticas/estatisticas.htm).

The objective of this study was the microbiological and chemical characterization of *alheiras*. Particular reference is made to factors which might influence the safe consumption of this food. The data presented will be valuable in exposure assessment exercises for a number of known chemical and microbiological hazards. The identification and the quantification of the properties that better describe the characteristics of this product are also important as *alheiras* are in the process of being given the status of a product from a protected denomination of origin.

Materials and methods

Alheiras vary considerably in their final compositions and production processes. The relevant common elements in the production process are the boiling of various meats in lightly salted and spiced water; soaking the thinly sliced bread in some of the broth, formed during the boiling of the meats, until it is soft enough; adding meat in small pieces, spices and olive oil and/or fat drippings to the bread/broth mixture; no adding of starter cultures; stuffing the paste into cattle intestinal or cellulose/based casings when everything is completely mixed and the salt and spices adjusted to the desirable taste (variable); smoking the formed horseshoe-shaped sausages (c.a. 15 cm long; ±60, for 2–8 days). The shelf life of *alheiras* is about 1 month if stored at 4 °C in air or longer if the sausages are vacuum packed. *Alheiras* are cooked before consumption either by frying, grilling or boiling, according to regional traditions or consumer preferences.

Sampling

Alheiras from 12 different producers were collected from retail establishments during the period September 2003–May 2004. With the exception of two specific producers, which were only sampled once, samples were collected on at least two different occasions in order to have samples from two different batches. Samples were transported to the laboratory in portable, insulated cold-boxes and stored at 4 °C until they were analysed, normally between 1 and 5 days after collection. From each batch, six *alheiras* were divided into various pieces. For each parameter to be evaluated, unless otherwise stated, 2–4 independent analyses were performed using randomly selected pieces.

Chemical analyses

General composition

The approximate 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 (1975a), 3091 (1975b), 1841-2 (1996a) and 1442 (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, USA).

Lead, cadmium, arsenic and mercury determination

Lead, cadmium, arsenic and mercury determination was by atomic absorption spectrometry. Arsenic was determined according to AOAC Methods (1997). Mercury was determined according to the European Standard EN 13806 (ECS, 2002). Cadmium and lead were determined according to the European Standard EN 14082 (ECS, 2003).

Biogenic amines determination

Ten grams of each sample of *alheira* were weighed into an 85 ml test tube and extracted with 20 ml of trichloroacetic acid (5%). Extracts were derivatized with *o*-phthalaldeyde (OPA) and biogenic amines were determined by HPLC using a method based on that described by Komprda et al. (2004).

Microbiological analyses

Twenty-five grams samples were added to 225 ml of sterile buffered peptone water (Merck, Darmstadt, Germany), and homogenized in a stomacher for 2 min. Appropriate decimal dilutions were prepared in Ringer solution (LabM, Bury, UK) for microbial enumeration: lactic acid bacteria on de Man, Rogosa Sharpe Agar (MRS, LabM) and 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; *Escherichia coli* on TBX (BioRad, CA, USA), 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 (2000); sulphite reducing *Clostridium* spores according to the Portuguese Standard NP 2262 (IPQ, 1986).

Enumeration of *Listeria* spp. was performed by the most probable number (MPN) technique using culture media referred to in ISO 11290-1 (1996b), namely Demi–Fraser broth, Fraser broth and Palcam medium. The MPN of *Listeria* spp. in each sample was determined using the FDA

online MPN table (www.cfsan.fda.gov/~ebam/bam-a2.html).

Results and discussion

Chemical analysis

The results of the physicochemical analyses are shown in Table 1. Generally, these indicated that, according to the generally accepted limits for these parameters, pH, salt content and humidity per se, do not assure the microbiological safety of this product. The meats in this product are boiled sufficiently to inactivate the vegetative pathogens, but post-process contamination can occur via the addition of the bread and spices and by subsequent handling during filling and later manipulations.

For all samples analysed, nitrate and nitrite concentrations were lower than the accepted limits for these parameters (European Commission, 1995), 250 and 100 mg/kg, respectively. It is noteworthy that, unlike many similar products, *alheiras* are not traditionally prepared using nitrate or nitrite and thus it is expected that the concentration of these compounds will be very low.

Lead, cadmium, arsenic and mercury

Maximum permitted levels of cadmium and lead in meats, as defined in Commission Regulation No. 466/2001, are 0.05 and 0.1 mg/kg, respectively. With the exception of one sample out of two analysed from producer 10 in which the lead concentration was 0.6 mg/kg, the legislated maximum value was never exceeded (Table 2). High lead concentrations in a Greek fermented meat product, 0.7750 mg/kg, have also been reported by the Directorate-General Health and Consumer Protection (2004).

There is no legislation in force controlling arsenic in foods within the EU. Levels found in the present study, with the exception of samples from producer 1 in which mean arsenic concentration of three different products was 0.061 mg/kg (Table 2), are in 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 the UK, 0.0037–0.033 mg/kg.

Table 1
Minimum, maximum, mean and standard deviation of physicochemical parameters and composition of *alheira*

	Min.	Max.	Mean	SD
pH	4.5	6.3	5.11	0.5
% NaCl	1.0	1.8	1.3	0.3
% Moisture	43.3	57.2	52.3	4.31
% Fat	10.9	29.6	18.4	4.7
% Total protein	6.9	15.5	11.4	2.8
% Carbohydrates	10.2	20.9	15.2	3.6
Energy (kcal/100 g)	220	369	274.4	39.7

There is no legislation in force controlling mercury in foods within the EU. Levels found in the present study, are in the range of values reported by the Directorate-General Health and Consumer Protection (2004) for meat and meat products produced in Denmark, France, Germany, Ireland and UK 0.0015–0.012 mg/kg (Table 2) well below the maximum level defined in Commission Regulation No. 466/2001 for bivalves, cephalopods and crustaceans, 0.5 mg/kg.

Biogenic amines

A great variability was observed in the biogenic amines content of the products from different producers and even from two batches of the same producer (Table 3). A high variability in the biogenic amines content was previously reported for sausages produced by different manufacturers (Tschabrun, Sick, Bauer, & Kranner, 1990) and for different batches of the same commercial products (Hernández-Jover, Izquierdo-Pulido, Veciana-Nogués, Mariné-Font, & Vidal-Carou, 1997). Tyramine, ethylamine and 2-phenylethylamine were the biogenic amines with the highest concentrations in the samples analyzed. Tyramine has been systematically reported as the most abundant amine in fermented sausages (Coïsson, Cerutti, Travaglia, & Arlorio, 2004; Komprda et al., 2004). The presence of 2-phenylethylamine only occurred when tyramine was also detected. This correlation was also observed by other researchers (Ansorena et al., 2002) and has been attributed to the non-specific activity of tyrosine decarboxylase (Joosten, 1988). The toxic level of tyramine is 100–800 mg/kg (Silla-Santos, 1996). Accordingly, and based on the present results, biogenic amines in *alheiras* apparently do not represent an obvious or generalised hazard.

Microbiological analyses

Concerning the microbiological status of the products, as shown in Tables 4 and 5, great variability was observed between producers and even between lots from the same producer. These might be the result of small differences in the processes, as *alheiras* from different localities were sampled and also because the age of the various samples is certainly different (and not known – they were obtained from the local retail market and sold "loose" so that neither the production date nor the "best before" date are labelled).

It is obvious that lactic acid bacteria (LAB) were the dominant microflora (Table 4). Counts on MRS and M17 were, in most cases, higher than 7.5 log cfu/g with the exception of samples from producer 6, where counts on MRS and M17 were 6.9–7.4 and 6.6–7.4 log cfu/g, respectively. With the exception of samples from producers 5 and 6, and one sample from producer 2, counts on mannitol salt agar were higher than log 6.5 log cfu/g. LAB and gram-positive, catalase-positive cocci have been demonstrated to have an important role in the manufacture of various traditional fermented sausages (Moretti et al.,

Table 2
Lead, cadmium, arsenic and mercury concentration in *alheira* (10^{-3} mg/kg)

	Producer											
	1	2	3	4	5	6	7	8	9	10	11	12
Lead	25 ± 20	40	19 ± 2	34	25	25	30 ± 3	23 ± 5	24 ± 6	298 ± 388	35 ± 14	32 ± 17
Cadmium	9 ± 2	10	8 ± 3	13	8	8	5 ± 1	7 ± 4	5 ± 1	7 ± 4	7 ± 1	7 ± 1
Arsenic	61 ± 14	<10	<10	<10	<10	<10	<10	8 ± 11	<10	<30	<30	<30
Mercury	<40	<50	<40	<50	<80	<80	<20	<40	<20	<20	<40	<40

Table 3
Biogenic amine content found in *allheira* (mg/kg)

Producer/lot	Histamine	Methylamine	Ethylamine	Tyramine	2-Phenylethylamine	Putrescine	Isoamylamine	Cadaverine
10/A	1.5	0.1	0	21.8	77.5	2.6	0.0	3.2
10/B	0.1	0.0	11.0	0.7	0.0	0.0	0.0	0.0
3/A	0.4	0.4	1.2	41.1	8.4	4.2	0.0	5.7
9/A	3.0	0.7	4.5	63.2	9.7	6.2	0.0	39.7
8/A	0.1	0.1	15.6	1.1	0.0	0.0	0.0	0.0
8/B	0.2	0.1	21.1	2.6	0.0	0.4	0.0	0.0

Table 4
Microbiological characterization of *alheira*: important microbial parameters in fermented products

Producer	Lot	Counts in MRS (log cfu/g)	Counts in M17 (log cfu/g)	Counts in MSA (log cfu/g)	Enterococci (log cfu/g)	Yeasts (log cfu/g)	Moulds (log cfu/g)
1	A	8.9 ± 0.1	8.8 ± 0.0	6.3 ± 0.0	6.5 ± 0.5	(ND)	(ND)
	B	5.9 ± 0.1	6.0 ± 0.0	6.3 ± 0	5.7 ± 0.0	2.9 ± 0.2	<1
2	A	8.8 ± 0.2	8.5 ± 0.1	4.3 ± 0.3	7.8 ± 0.1	(ND)	(ND)
	B	8.6 ± 0.0	7.6 ± 1.8	>6.2	8.1 ± 0.5	5.8 ± 0.1	<1
3	A	7.9 ± 0.0	7.7 ± 0.1	5.8 ± 0.3	6.8 ± 0.1	6.2 ± 0.1	5.5 ± 0.1
	B	9.0 ± 0.1	9 ± 0.1	>6.2	>7.2	6.1 ± 0.2	3.4 ± 0.0
4	A	7.7 ± 0.0	7.9 ± 0.0	5.9 ± 0.1	7.0 ± 0.1	3.9 ± 0.2	3.2[a]
	B	7.5 ± 0.3	7.6 ± 0.2	5.9 ± 0.1	4.5 ± 0.3	4.0 ± 0.72	<1
5	A	7.7 ± 0.4	7.5 ± 0.5	<1	6.7 ± 0.5	3.7 ± 0.3	1.8 ± 0.1
6	A	7.0 ± 0.3	7.0 ± 0.3	<1	4.3 ± 0.4	3.4 ± 0.3	1.8 ± 0.2
7	A	8.9 ± 1.3	8.9 ± 1.2	6.0 ± 0.2	7.1 ± 0.1	5.2 ± 0.9	3.7 ± 0.7
	B	8.1 ± 0.1	9.4 ± 0.1	>6.2	7.2 > 0.1	3.8 ± 0.0	2.3 ± 0.5
8	A	8.4 ± 0.2	8.4 ± 0.1	6.2 ± 0.1	6.9 ± 0.1	5.6 ± 0.1	2.9 ± 0.8
	B	8.0 ± 0.0	9.2 ± 0.3	>6.2	7.2 ± 0.1	4.2 ± 0.1	<1
9	A	8.9 ± 0.1	9.0 ± 0.1	5.8 ± 0.3	>7.2	6.2 ± 0.1	3.6 ± 0.2
	B	9.9 ± 0.2	>10.5	>6.2	>7.2	5.4 ± 0.1	1.3[a]
10	A	8.1 ± 0.0	8.6 ± 0.0	6.1 ± 0.6	6.3 ± 0.1	6.2 ± 0.1	4.2 ± 0.4
	B	7.8 ± 0.0	9.0 ± 0.1	7.2 ± 0.1	8.1 ± 0.2	3.4 ± 0.3	2.3 ± 0.4
11	A	8.9 ± 0.1	8.8 ± 0.1	5.9 ± 0.1	>7.2	3.5 ± 0.1	3.7 ± 0.5
	B	8.7 ± 0.1	8.8 ± 0.1	>7.2	>7.2	<1	2.1[a]
12	A	8.1 ± 0.1	8.1 ± 0.0	>6.2	> 7.2	6.4 ± 0.2	4.3 ± 0.9
	B	9.4 ± 0.0	9.2 ± 0.1	>7.2	>8.2	5.7 ± 0.5	5.6 ± 0.2

ND, not determined.
[a] Value obtained for one independent sample. Not detected in the other sample.

2004; Papamanoli, Kotzekidou, Tzanetakis, & Litopoulou-Tzanetaki, 2002; Papamanoli, Tzanetakis, Litopoulou-Tzanetaki, & Kotzekidou, 2003).

In addition to LAB and members of the Micrococcacae, yeast and moulds also play an important role in the development of the organoleptic characteristics of fermented

Table 5
Microbiological characterization of *alheira*: commonly controlled microbial safety parameters

Producer	Lot	*E. coli* (log cfu/g)	*S. aureus* (log cfu/g)	*Listeria* spp. (MPN/g)	S R C spores[e] (g)	Enterobacteria (log cfu/g)
1	A	(ND)	<1	2.3[b]	(ND)	2.6 ± 0.4
	B	2.5 ± 0.05[a]	<1	<0.2	(+)1[c]	2.9[c]
2	A	(ND)	<1	4.56[b]	(ND)	6.3 ± 0.2
	B	6.0 ± 0.2	4.1[c]	>240	(−)/1	6.9 ± 0.2
3	A	5.3 ± 0.3	3.6 ± 0.2	>240	(+)1	7.2 ± 0.4
	B	>7.5	<1	10.9 ± 0.0	(−)/1	>7.5
4	A	3.0 ± 0.6	<1.0	<0.2	ND	5.6 ± 0.2
	B	2.7 ± 0.3[a]	3.7[c]	17.6 ± 15.0[d]	(+)1	3.9 ± 0.9
5	A	<1	<1.2	1.8 ± 1.8	(−)/1	2.7 ± 0.4
6	A	2.5 ± 0.2	3.7 ± 0.1[d]	0.7 ± 0.4	(+)0.01	2.9 ± 0.0
7	A	>5.5 ± 1.2	3.0 ± 0.1[a]	127.1 ± 113.1	(−)/1	5.9 ± 1.6
	B	<1	<1	<0.2	(−)/1	3.3 ± 1.3
8	A	< 1	3.5 ± 02	(ND)	(+)0.1	6.3 ± 0.4
	B	3.7 ± 0.1	2.5 ± 0.3	(ND)	(+)1	5.5 ± 0.5
					(−)/1	
9	A	3.1 ± 0.5	2.7 ± 0.3[d]	>220.3 ± 34.2	(+)0.1	>7.5
	B	3.9 ± 0.05	4.5 ± 0.5	(ND)	(+)1	6.4 ± 0.7
					(−)/1	
10	A	4.9 ± 1.2	<1	(ND)	(+)0.1	>7.5
	B	6.3 ± 0.2	4.2 ± 0.4	(ND)	(+)1	6.6 ± 0.2
					(−)/1	
11	A	2.8 ± 1.2[d]	3.0 ± 0.0[a]	(ND)	(−)/1	7.3 ± 0.1
	B	6.2 ± 0.1	4.7 ± 0.2	<0.2	(−)/1	6.6 ± 0.1
12	A	<1	3.8[b]	>240	(−)/1	6.3 ± 0.7
	B	>6.5	<1	(ND)	(−)/1	>7.5

ND, not determined.
[a] Media of two independent samples. Not detected in the two other independent samples.
[b] Value obtained for one independent sample. Not detected in the other sample.
[c] Value obtained for one sample. Not detected in the other three samples.
[d] Media of three independent samples. Not detected in the other independent sample.
[e] Analyzed in 0.01, 0.1 and 1 g; (+), positive in; (−), negative in.

sausages (Mauriello, Casaburi, Blaiotta, & Villani, 2004). Yeasts and moulds were isolated from most of the products ranging from <1 log to 6.4 log and from <1 log to 5.6 log cfu/g, respectively.

Enterococci counts were higher than 6.5 log cfu/g in most of the products. Strains of this genus are frequently isolated from fermented sausages, especially in high pH products where no competitive starter cultures are used (Hugas, Garriga, & Aymerich, 2003). The metabolic activity of enterococci in the fermenting sausage matrix have not been studied in detail, however, they certainly contribute to sausage aromatization by their glycolytic, proteolytic and lipolytic activities (Sarantinopoulos et al., 2001). The presence of enterococci in foods however, is a concern, as many strains possess virulence traits (Franz, Stiles, Schleifer, & Holzapfel, 2003).

Concerning the indicator organisms investigated, it can be inferred that most of the *alheiras* were produced under deficient hygienic conditions leading to post-process contamination after boiling of the meats (Table 5). It should be pointed out that according to the guidelines for the microbiological quality of fermented meats published by Gilbert et al. (2000), most of the samples tested would be considered unsatisfactory as Enterobacteriaceae and *E. coli* counts were higher than 4 log and 2 log cfu/g, respectively. The detection of *Listeria* spp. in some samples, an important indicator of plant hygiene, due to their ubiquitous nature, points to the possibility of product contamination with the pathogenic species *L. monocytogenes*. According to these guidelines, four samples should be considered as unacceptable/potentially hazardous as coagulase-positive staphylococci were found in a concentration higher than 4 log cfu/g (Gilbert et al., 2000). In general, the results obtained have shown that the optimization of hygienic procedures in the production process is necessary to improve the quality and safety of *alheiras*.

Further work will be carried out in order to evaluate the incidence of specific pathogens and in order to identify the main sources of product contamination.

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