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PREFACE

It is our pleasure to introduce you **The International Food Congress** entitled **"Novel Approaches in Food Industry"** which will be held in Çeşme, Izmir, TURKEY. The congress will take place on 26-29 May, 2011 and include a variety of hot topics such as novel food products and technologies, thermal and non-thermal food processing technologies, applications of nanotechnology in food processing, innovations in food science and technology. This congress will highlight the most important areas of recent Research & Development in Food Science and Technology as well as explore relevant and interesting topics for the future. The congress will also provide accurate and updated scientific information and trends for the discipline of food science and technology. 400 leading scientists from all over 40 countries will contribute to the congress as oral or poster presentations.

This congress will provide a forum for the exchange of ideas and authoritative views by leading scientists, as well as business leaders and investors in the food industry. **More than 32 leading food industry companies became sponsor or supporting organization to our congress.** Outstanding keynote speakers and well-known leading scientists and experts from around the world will be sharing their knowledge with us. Company executives, as well as speakers from universities, research centers and governmental institutions will discuss scientific and technical developments in detail.

We would like to thank all contributors including authors of oral and poster presentations and our sponsors for contributing to the success of this congress.

On Behalf of the Executive Committee

Prof. Dr. Sebnem TAVMAN

Microbiological characterization of *alheira*, a typical Portuguese fermented sausage, and its relation with hygienic conditions of the processing environments

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Abstract

Fermented meat products are part of the daily diet in rural areas of Portugal. Nevertheless, in urban centers the demand for this kind of products has been increasing. *Alheiras* are traditional, smoked, naturally-fermented meat sausages, produced in the North of Portugal and generally in kitchens of small dimensions, known as regional kitchens.

In this study three regional kitchens of *alheiras*, located in the North of Portugal, were evaluated in terms of microbiological quality of the environment – surfaces, equipment and ambient air -, handlers and food product, in two distinct periods: Period I – immediately after preparation and filling of sausage, and Period II – after five days of drying.

In terms of microbiological quality of the surrounding air, poor hygienic conditions were often found for food preparation. Microorganisms were found only in two areas of surface equipment and utensils, namely, coliforms and *E. coli*. These results revealed poor hygienic practices. Microbiological analysis of the hands of food operators showed that only one person had unsatisfactory results. In relation to *alheiras*, these did not present any danger to consumer health. Nevertheless, some high scores were detected in relations to mesophiles, which might decrease the quality of the product. It must be highlighted that microorganisms, such as *Salmonella*, *Listeria monocytogenes* and *S. aureus*, associated with foodborne diseases were not detected.

Key words: *Alheira*, regional kitchens, processing conditions, hygiene, safety.

Introduction

Alheiras are traditional, smoked, naturally-fermented meat sausages, produced in the North of Portugal. These products are part of the daily diet in rural areas of Portugal and are considered fashionable food products in urban centers whose market has been increasing in a significant way (Ferreira *et al.*, 2006). The raw materials used in the manufacturing of sausages are pork meat, regional wheat bread, olive oil and seasoning salt, garlic and paprika. The production of these products is carried out generally in kitchens of small dimensions, known as regional kitchens. These kitchens are small units that cannot produce more than 2000 kilograms of sausages per year and can only market the products within a maximum radius of 40 km (Ferreira *et al.*, 2007a).

Several studies have been carried out in Portugal in order to infer the quality and safety of these products. The works performed by Ferreira *et al.* (2006) and Patarata *et al.* (2008) have corroborated the fact that we are dealing with products whose characteristics, such as water activity and pH, may ensure stability at room temperature. However, these products have been repeatedly associated to the presence of pathogenic bacteria such as *Listeria monocytogenes*, *E. coli* O157:H7, *Satphylococcus aureus*, *Salmonella* spp., *Campylobacter*, *Clostridium botulinum* and *Clostridium perfringens* (Ferreira *et al.*, 2007a, 2007b) which have indicated poor manufacturing practices and inadequate hygiene. In order to control contamination of the processing environment and products, it is important to detect the sources of contamination and true critical points. Handlers and surfaces of processing equipment have been recognized as sources of microbial contamination.

In this context, the objective of this study was to evaluate the microbiological quality of the environment – surfaces, equipment and ambient air -, handlers and food products in three regional kitchens where *alheiras* are produced, in two distinct periods: Period I – immediately after preparation and filling of sausage, and Period II – after five days of drying.

Material and Methods

Sampling

Alheiras from three regional kitchens were collected from retail establishments from December 2009 to March 2010. Samples were collected in two distinct periods: Period I – immediately after preparation and filling of sausage, and Period II – after five days of drying. This time is considered by the producers as sufficient to adequately dry/smoke and allow the *alheira* to be ready for consumption.

Twenty two environmental surface samples from mincing, mixing and stuffing machines, cutting tables, deboning knives and cutting bread machines were collected in each sampling period, according to ISO 18593:2004, after the cleaning and disinfection procedures routinely followed by each producer. In order to perform sample collections, sterile swabs dampened in peptone water were used. Areas of 25 and 100 cm^2 were analyzed.

The microbiological air quality was evaluated by the simple sedimentation technique. Air samples were taken in the processing zone, drying zone and exterior of the building. In order to do this, Petri dishes with medium were left opened for 15 minutes.

The right and left hands of food handlers were analyzed by using sterile swabs moistened in peptone water that were passed over the entire surface of the hands and between the fingers.
All samples were transported to the laboratory in portable, insulated cold-boxes and stored at 4°C until analysed.

Microbiological analyses

Twenty-five grams of samples were added to 225 ml of sterile buffered peptone water and homogenized in a stomacher for 2 min. Appropriate decimal dilutions were prepared in peptone water for microbial counting. The media and the conditions of incubation were the following for the microorganisms: Mesophiles: Plate Count Agar (PCA, Oxoid) incubated at 35°C for 72h; Yeasts and molds: Potato dextrose agar (PDA, Oxoid) with 0.1 g/L of chloramphenicol, incubated at 25°C for 5 days; *Staphylococcus* positive coagulase: Baird-Parker medium enriched with egg yolk with tellurite, incubated for 48 h at 37°C. Typical colonies of *S. aureus* were tested for coagulase reaction; Total coliforms and *Escherichia coli*: SimPlate method (Biocontrol®) incubated at 37° C for 24 h; *Listeria* spp: Imunoprecipitation method (VIP Listeria, Biocontrol®); Salmonella: 1-2 Test (Biocontrol®), with a pre-enrichment in 225 ml of Buffered Peptone Water (Oxoid) for 16-20 h at 35–37°C.

The results obtained in this study were analysed on the basis of the microbiological guidelines for food ready to eat reported by Santos (2005).

For the counting of the total number of mesophiles and yeast and molds present in the air, the Petri dishes with PCA were incubated for 24–48 hours at 37°C for mesophiles, and the Petri dishes with PDA for 5 days at 25°C for yeast and molds. Results were expressed on colony forming units per cm^2 and week (cfu/cm^2/week).

Statistical analysis

All microorganisms' counts were transformed to $\log_{10}$ values using the JMP Software. The significance of the differences found between the counts was evaluated by Tukey's test. Differences were considered significant at $p < 0.05$ level.

Results and Discussion

The counting of aerobic mesophilic bacteria and molds and yeasts determined in the air environment, after preparation and filling of sausage (Period I) and after drying (Period II), are presented in Table 1. Generally, the numbers of mesophilic aerobic bacteria and yeasts and molds in the drying zone were higher than in the processing zone. These results could be due to the processing activity that takes place for a shorter period of time than the activity of drying the sausages, which are smoked for at least five days. On the other hand, operators do not always prepare sausages daily, because this activity depends on the availability of raw materials and demand. Thus, many times the preparation room is not used, remaining clean and disinfected which results in a smaller number of mesophiles and yeasts and molds in relation to the drying room.

According to the American Public Health Association (APHA), for an environment to be considered satisfactory in terms of hygiene conditions, it must present a count of aerobic mesophiles less than 30 $cfu.cm^{-2}.week^{-1}$. Considering the same number for yeasts and molds, the present results indicate that the microbiological quality of the ambient air of the three regional kitchens studied were indicative of poor hygienic conditions.

Table 1. Counts of aerobic mesophiles and molds and yeasts ($cfu.cm^{-2}.week^{-1}$) at the processing areas, drying zones and exteriors of three regional kitchens, immediately after preparation and filling of sausages (Period I) and after the 5-days drying period (Period II).

Kitchen	Ambient	Period	Mesophiles			Molds and Yeasts		
			Min[a]	Med[b]	Max[c]	Min[a]	Med[b]	Max[c]
A	Processing zone	I	10.55	31.65	42.20	0	15.83	31.65
		II	0	31.65	42.20	31.65	52.75	116.05
	Drying zone	I	10.55	36.93	94.95	0	15.83	31.65
		II	31.65	63.30	94.95	52.75	79.13	147.70
	Exterior	I	116.05	>3165	>3165	464.20	485.30	>3165
		II	0	5.28	21.10	42.20	89.68	221.55
B	Processing zone	I	0	15.83	31.65	31.65	42.20	84.40
		II	10.55	47.48	94.95	10.55	36.93	94.95
	Drying zone	I	31.65	79.13	94.95	42.20	47.48	94.95
		II	31.65	58.03	73.85	73.85	116.05	137.15
	Exterior	I	0	5.28	21.10	42.20	58.03	189.90
		II	31.65	42.20	84.40	84.40	137.15	147.70
C	Processing zone	II	52.75	205.73	242.65	31.65	216.28	>3165
	Drying zone		137.15	390.35	527.5	10.55	184.63	379.80
	Exterior		126.60	152.98	>3165	42.20	142.43	316.50

[a] – Minimum, [b] – Median, [c] – Maximum

In relation to the hygienic conditions of equipment and utensils (Table 2), mesophiles counts were above 1 cfu/cm^2 on a filling counter in kitchen C (Period II), filling machine in kitchen A (both periods) and bread slicing machine in kitchen C (Period II); however, these values are not considered unsatisfactory by Forsythe and Hayes (2002), which only consider values above 100 cfu/cm^2 to be unsatisfactory. Applying this criterion to molds and yeasts, only the chopping machine in kitchen B (Period I) had an unsatisfactory level of sanitation (> 100 cfu/cm^2).

In terms of total coliforms counts, they were only high in the filling machine (2 cfu/cm^2) of kitchen A in both periods (I and II). *E. coli* was not found, except on one knife of kitchen B (10 cfu/cm^2). This value seems quite high and worrisome, when compared with the value suggested as acceptable for

enterobacteria (0-1 cfu/cm^2) for working surfaces and cutting plants, referred in the Commission Decision 2001/471/EC of 8th June. In relations to *S. aureus*, it was not detected.

Table 2. Counts of mesophiles, molds and yeasts, total coliforms, *E. coli* and *S. aureus* (cfu/cm^2) determined on some equipment and utensils present in the three regional kitchens in Periods I and II.

Surface	Counter Filling			Filling machine				Chopping machine	Bread slicing machine				Knife			
kitchen	A		C	A		B	C	B	A		B	C	A		B	C
Period	I	II	II	I	II	I	II	I	I	II	I	II	I	II	I	II
Mesophilic	<1	<1	**1.3**	**6**	**6**	<1	<1	<1	<1	<1	<1	**3.3**	<1	<1	<1	<1
Molds and yeasts	<1	<1	<1	<1	**2.8**	**9.8**	<1	**>100**	<1	<1	<1	<1	<1	<1	<1	<1
Coliforms	<1	<1	<1	**2.0**	**2.0**	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
E. coli	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	**10**	<1
S. aureus	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1

Generally, these results point out the need for making prompt corrections in the sanitation practices of some working surfaces and utensils, since these are in direct contact with food, probably causing its contamination.

Regarding the hands of food handlers, only positive results (2 cfu/sw), were determined in the operator of kitchen A in regards to coliforms and *E.coli*, in Period II. All other food handlers had properly cleaned hands, as the presence of *S. aureus*, coliforms and *E. coli* was not detected.

The results of the microbiological analyses of *alheira* are shown in Table 3. Total mesophiles and yeasts and molds counts had almost doubled in the Period I to Period II, in kitchens A and B. In relations to kitchen C, the values of yeasts and molds were similar to those found in the other two kitchens in Period II. However, the values of mesophiles determined in the former were the highest. High counts of total mesophiles and yeasts and molds in sausages were also observed by Esteves (2005) and Esteves *et al.* (2006), 8,28 log cfu/g and 8 log cfu/g, respectively. According to these authors, these values are indicative of poor microbiological quality of products. It should be noted that the highest level of yeasts and molds determined in *alheiras* of kitchen B, in Period I, may be related with the highest counts found in the filling machine (9.8 cfu/cm^2) and chopping machine (>100 cfu/cm^2), whose values were previously discussed.

Taking into account the microbiological limits established by Santos *et al.* (2005) for ready to eat foods of Group 2, where the fermented sausages are included, namely, mesophiles: $\geq$ 5 log cfu/g, yeasts: $\geq$ 4 log cfu/g, molds: $\geq$ 2 log cfu/g; coliforms $\geq$ 3 log cfu/g and *E. coli* $\geq$ 1 log cfu/g, the sausages analysed were found to have an unsatisfactory microbiological quality. However, these results should be viewed with some caution because the sausages analysed in the present study had not undergone any type of thermal processing.

The presence of *E. coli* in fermented meat products has been reported in other studies. 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 Table 3, *E. coli* was only detected in *alheiras* of kitchen A in Period II. Neither *Listeria* spp., *S. aureus*, nor *Salmonella* spp. were detected in any *alheira* sample. On the other hand, the presence of these microorganisms had been reported by Esteves *et al.* (2006) and Ferreira *et al.* (2007a; 2007b) in *alheiras* from industrial producers.

Table 3 - Microbiological quality of *alheiras* (log cfu/g) (mean ± standard deviation) prepared in three kitchens during Periods I and II.

Kitchens	Period	log cfu/g			
		Mesophiles	Molds and yeasts	Coliforms	*E. coli*
A	I	2.6±0.2	2.5±0.5	1.1±0.2	<1.0
	II	4.8±3.1	7.6±1.4	2.1±1.5	1.1±0.2
B	I	4.7±0.7	4.8±0.6	1.4±0.7	<1.0
	II	7.0±1.1	8.4±1.0	1.1±0.1	<1.0
C	II	8.5±0.6	8.1±0.3	2.9±0.5	<1.0

Conclusions

The microbiological quality of the environment found in the regional kitchens is frequently unsatisfactory for food processing. In relations to the contamination of surfaces and equipment, we found microorganisms (coliform and *E. coli*) indicative of poor hygiene practices. Microbiological analysis to the hands of food handlers showed that unsatisfactory results were only observed in one person.

The present study also showed that the fermented sausages produced in the regional kitchens did not present a danger to consumer health, despite high counts of mesophiles having been detected. These may decrease the microbiological quality of the product. It should be noted that microorganisms associated with foodborne illnesses were not detected.

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