



**CECAV**  
ANIMAL AND VETERINARY  
RESEARCH CENTER

---

## CECAV-UTAD Publications

Institutional Repository of the  
University of Trás-os-Montes and Alto  
Douro

Found at: <http://repositorio.utad.pt/>

This is an author produced version of a Book  
Chapter published in Mediterranean Ecosystems:  
Dynamics, Management and Conservation. Williams,  
G.S. (Ed.), Nova Science Publishers Inc

This Book Chapter has been peer-reviewed  
but does not include the final publisher  
proof-corrections or publisher pagination.

Talon, R., Lebert , I., Lebert , A., Leroy , S., Garriga,  
M., Aymerich, T., Drosinos, E.H., Zanardi, E., Ianieri,  
A., Fraqueza, M.J., Patarata, L., Laukova, A., 2012.  
Microbial ecosystem of traditional dry fermented sausages  
in Mediterranean countries and Slovakia. In: Mediterranean  
Ecosystems: Dynamics, Management and Conservation.  
Williams, G.S. (Ed.), Nova Science Publishers Inc.,  
Hauppauge NY, pp. 115-127.

Published in final form at:

<https://www.novapublishers.com>

Copyright: Nova Science Publishers Inc.

Access to the published version may  
require subscription.

Key words: traditional dry fermented sausages, small processing unit, microbial ecosystem, environment.

# 1.Introduction

Since 1980's, traditional food production has gained a growing interest overall Europe. As part of the common agricultural policy, the diversification and the promotion of food products having certain characteristics, such as an identifiable geographical origin or ancient manufacturing practices, is encouraged due to the considerable benefits to the rural economy, in particular for less favoured or remote areas (Council Regulation (EC) 510, 2006; Council Regulation (EC) 509, 2006).

Due to different so-called food crises, food safety has become a hot topic in the media and has raised a wide range of concerns among consumers and therefore, in many European countries, the demand for traditional food products has increased (Wilcock, Pun, Khanona, & Aung, 2004; Röhr, Lüddecke, Drusch, Müller, & Alvensleben, 2005). In addition, food and gastronomy form an inherent link with tourism in Europe and, recently, there has been a renewed interest by some groups of consumers in typical and regional food, made in a non-industrial environment, characterized by small-scale batch production with a limited degree of mechanization and strongly identified with a place or region of origin (Kuznesof, Tregear, & Moxey, 1997).

Within the sector of processed meat, traditional production can be classified in two main types. The first one is a farm production in which raw meat is obtained from animals bred in the farm (using in some regions autochthons bred animals) and processed either in the farm or in very small collective production units, and the resulting products are sold directly by the farmer. This system is well-developed in France, Germany, Belgium, United Kingdom. (Reference ?). It is also present in Italy, Slovakia and Northern Portugal. The second one is a production by local processing units that could be or not owned by a butcher that manufactures traditional products from raw meat coming from several farms. In Portugal raw materials could come from local slaughterhouses. The products are sold directly by the unit at the local market or supplied to restaurants and delicatessen. This production is developed in South of France, Spain, Italy, Portugal and in Germany (Conter, Zanardi, Ghidini, Pedrelli, Rason, & Chizzolini, 2005).

Dry fermented sausages, mainly manufactured with pork and/or beef lean and pork fat, account for a significant part of traditional meat products. Most of these products seasoned and processed with particular know-how rely on raw material natural contamination that occurs during animal slaughtering and increases during manufacturing steps. In traditional dry fermented sausages, this contaminating microflora colonises processing unit's environment and the products in a continuous symbiotic exchange. Such microflora includes useful microorganisms for the fermentation and flavour of sausages but also spoilage species and, sometimes, pathogenic bacteria. Many studies concern the microbiology of the traditional fermented sausages (Aymerich, Martín, Garriga, & Hugas, 2003; Papamanoli, Tzanetakis, Litopoulou-Tzanetaki, & Kotzekidou, 2003; Mauriello, Casaburi, Blaiotta, & Villani, 2004; Comi et al., 2005; Rantsiou et al., 2005) but only two studies in France focused on the microbiology of surfaces and equipment of processing units (Corbiere Morot-Bizot, Leroy, & Talon, 2006; Chevallier et al., 2006).

The European project TRADISAUSAGE (QLK1 CT-2002-0224) aimed to evaluate and improve the safety of European traditional dry fermented sausages manufactured in small processing units, from the producers to consumers while preserving their typical sensory traits. The processing units investigated were all low-capacity establishments of meat products according to the definition of the Council Directive 92/5/EEC on health problems which defines as low-capacity establishments those not having an industrial structure and

having a total production capacity less than 7.5 ton/week. In the framework of this project some tasks dealt with the study of overall microbial characterization both of the surfaces and equipments (the present paper) and of the meat products at three steps of production (Talon et al., paper 2, 2007).

This paper presents a review of the microbial ecosystem of environments in 54 processing units located in five Mediterranean countries (Spain, Italy, France, Greece, Portugal) and one from east of Europe (Slovakia). The objective was to analyse the diversity of the residual spoilage, technological and pathogenic microflora contaminating the surfaces and equipments. Thus the microflora was analysed on six environmental surfaces in 54 processing units in order to control the hygienic practices.

## **2. Materials and methods**

### **2.1. Processing units**

The 315 traditional processing units (PUs) enquired (data not shown) were located in European Mediterranean countries and Slovakia and represented a large diversity of economic characteristics (Table 1). More than 50% belonged to “very small enterprises” category because they employed less than ten people, two or three in most of the cases. Processing units were more numerous in the mountainous regions: e.g. Massif-Central of France, Alentejo of Portugal.

The 315 PUs were clustered according to their significant characteristics involving raw material and processing criteria. Ten representative PUs per country (five for Slovakia) were selected using the method of Rason et al. (2004). The microbial diversity in the environment was studied in the 54 selected PUs (Table 1).

### **2.2. Sampling procedure**

Sampling and analytical methods and microbial analysis were harmonized among the research teams participating in the project and according to International Standards (ISO).

Six environmental surface samples were collected according to ISO 6887-1 (1999) in each PU: three from the machines (mincing, mixing, stuffing), one from the cutting table, one from the wall of storage cold room and one from the deboning knives. Samples were collected after cleaning and disinfection procedures and before manufacturing began. Samples representing 500 cm<sup>2</sup> of the environmental surface were collected using a swabbing technique performed with a sterile cloth dampened with a neutralizing solution (Humeau, La Chapelle-sur-Erdre, France). Sterile wet clothes were aseptically transferred to 25 ml of sterile buffered peptone water solution (AES Laboratory) and homogenised with a stomacher.

### **2.3. Microbial enumeration**

Occurrence or enumeration of the following bacteria - *Pseudomonas*, yeasts and moulds, lactic acid bacteria (LAB), *Staphylococcus* and *Kocuria*, *Enterobacteriaceae*, enterococci, *Staphylococcus aureus* and coagulase positive staphylococci, *Listeria monocytogenes*,

*Salmonella*) were performed with the methods presented by Lebert et al. (2007) and according to ISOs (Table 2).

## 2.4. PCR analysis for pathogenic bacteria

Some PCR protocols were established according to the methodology described in EU “Food PCR” project (QLK1-CT199-00226) and from the literature (Table 3).

PCR amplifications of specific fragments were used to analyse potential *Salmonella* from Brilliant-green Phenol-red Lactose Sucrose agar plates (Merck), potential *L. monocytogenes* from *Listeria* agar according to Ottaviani & Agosti (ALOA, AES Laboratory) plates and *S. aureus* from Baird Parker supplemented with Tellurite Yolk Egg or Baird Parker +RPF (Table 3). An isolated colony was suspended in 30 µl of sterile distilled water and 2 to 5 µl were added to the PCR reaction mix according to Lebert et al. (2007).

To detect the presence of *L. monocytogenes*, enrichment solution was analysed (Lebert et al., 2007). A Chelex DNA extraction was applied before amplification as followed: one ml of enriched culture was spined and the pellet was resuspended in 300 µl 6% Chelex-100 (Bio-Rad, Marnes-la-Coquette, France). The suspension was vortexed roughly for 10 s and incubated at 100°C for eight min. The tube was vortexed roughly 10 s and chilled on ice two min. Supernatant containing purified DNA was obtained after a centrifugation 5 min at 10000 g at 4°C. 5 µl were used as template DNA in the PCR reactions.

## 2.5. Statistical analysis

### Principal component analysis

Principal component analysis is a mathematical method used to reduce the dimensionality of the data set while retaining as much information as possible. It computes a compact and optimal description of the data set. The first principal component is the combination of variables that explain the greatest amount of variation. The second principal component, and subsequent, defines the next largest amount of variation and is independent to the first principal component. The number of significant principal components indicates the number of fundamentally different properties exhibited by the data set. This number is chosen from the evolution of the eigenvalues in the Scree plot and for eigenvalues higher than one. PCA was applied with the PCA routine in the Statistica computer program (Statistica version 6.1, Statsoft inc., Maisons-Alfort, France).

A PCA was performed to analyse the repartition of the microflora in the environment among 53 processing units of the six countries. The six following microflora were considered: yeasts and moulds, lactic acid bacteria, *Staphylococcus/Kocuria*, *Enterococcus*, *Enterobacteriaceae*, *Pseudomonas*. Six surface samples were considered: mincing, mixing and stuffing machines, table, cold room and knives.

### Multiple analysis of variance and Newman-Keuls tests

A multiple analysis of variance was used to analyse the main and interaction effects of two independent variables (countries, sampling) on the dependent variables (the six microflora). The "main effect" is the direct effect of the independent variable on the dependent variable. The "interaction effect" is the joint effect of the two independent variables on the dependent variable. The key statistic in multiple analyses of variance is the F-test of difference of group means, testing if the means of the groups formed by values of the independent variable are different enough not to have occurred by chance. If the group means do not differ

significantly then it is inferred that the independent variables did not have an effect on the dependent variable. If the F test shows that overall the independent variables is related to the dependent variable, then multiple comparison tests of significance using a Newman-Keuls method, are used to explore just which values of the independents have the most to do with the relationship. Statistical significance was judged at the 5% level.

ANOVA was calculated with the ANOVA routine by Statistica software (Statistica version 6.1, Statsoft inc., Maisons-Alfort, France).

## 3. Results

### 3.1. Pathogenic microflora

On the 314 surface and equipment samples analysed, *Salmonella* was detected in 4.8% (Table 4). 14 samples out of 15 were detected in Greek PUs, these samples mainly arose from knives, cold rooms, mincing and mixing machines of different PUs. *S. aureus* was enumerated in 6.1% of the samples and was found on the different surfaces in five countries out of six studied. *L. monocytogenes* was enumerated in 2.2 % of the samples and in four countries. PCR after enrichment led to the detection of *L. monocytogenes* in ten further samples.

### 3.2. Other microflora

A principal component analysis was applied to the microbial data of the 53 PUs of the six countries. From the eigenvalue results (data not shown), two principal components were chosen and accounted for 79.7% of the total variation of the initial data set. Figure 1a shows a high significance of principal component 1 that explained 62.1% of the total data variance. This axis was influenced by almost all the microbial groups in only one direction (negative values). Principal component 2 (17.6%) was influenced by yeasts and moulds. In Figure 1(b), principal component 1 separated the PUs according to the level of residual contamination in the environment. Most Italian PUs were characterised by low contamination whatever the microbial groups and the surface samples. But when contaminated, surfaces showing greater contamination were mainly the knives. Processing units from France, Portugal and Spain were gathered having a wide range of contamination levels – from low to high contaminated ones. When samples were heavily contaminated, they were also contaminated by yeasts and moulds (principal component 2). In France, two processing units were particularly contaminated indicating insufficient cleaning and disinfection operations. Cold rooms and mixing machines were generally the less contaminated while knife and table samples were the most contaminated. In Portugal, the microorganisms that had higher counts were yeasts and moulds, always present in samples, even with a low counting. The maximum value of that microflora was 6.1 log CFU/100 cm<sup>2</sup>. *Pseudomonas* spp. was also found in high amount in several samples. Its counts ranged from the limit detection to 7.6 log CFU/100 cm<sup>2</sup>. In Spain, the surfaces showing great contamination were stuffing and mincing machines and cutting tables. Greek processing units showed also a wide range of microbial contamination. In these PUs, surface samples characterised by high contamination were associated with high enterococci and low yeasts and moulds levels. The surfaces showing great contamination were knives, mincing machines and cold rooms. But one Greek processing unit was characterised by the highest counts of all the investigated microorganisms. Slovak processing units had intermediate level of contamination when compared to Greek group and France-Portugal-Spain group.

The multifactorial analysis of variance shows that the country variable had a highly significant effect on all the microbiota (Table 5) while the samplings had only a significant effect on the enterococci, *Enterobacteriaceae* and *Pseudomonas*. The interaction between the country and sampling variables had only a significant effect on the *Enterobacteriaceae* and LAB (Table 5).

Concerning the main effect of the country, Newman-Keuls tests showed that the Italian PUs presented the lowest level of microbial contamination, inferior to 1.0 logCFU/100 cm<sup>2</sup> (Table 6). On the opposite, the Greek PUs had the highest contamination for all the microbial populations, ranging from 3.5 to 5.0 logCFU/100 cm<sup>2</sup>, except for yeasts and moulds where the level was the lowest. Intermediate levels of contamination were noticed for the PUs from France, Spain, Portugal and Slovakia. However, the PUs from Slovakia can be distinguished on the basis of their low level of yeasts and moulds and *Pseudomonas* (Table 6). Concerning the effect of samplings, cold rooms and mixing machines were always the least contaminated samples with level ranging from 1.0 to 2.5 logCFU/100 cm<sup>2</sup> (Table 7). The four other surface samples (stuffing and mincing machines, knives, tables) were close in contamination, but in general the tables and the knives were the most contaminated with levels ranging from 2.2 to 3.3 log cfu/100 cm<sup>2</sup>. The contaminations of the surfaces were higher for *Staphylococcus/Kocuria* and *Pseudomonas*.

## 4. Discussion

Meat or fat residuals may not be removed totally if an insufficient cleaning procedure is applied. Residuals may act as vehicles for spoilage or pathogenic bacteria from surfaces to meat (Gill & McGinnis, 2004). Consequently unclean, insufficiently or inadequately cleaned pieces of equipment have often been identified as the source of pathogens (Reij & Den Aantrekker, 2004). Many studies investigated the pathogen flora of food-processing environments and food processing lines such as *Escherichia coli* in a beef-packing plant (Aslam, Greer, Nattress, Gill, & McMullen, 2004), *Listeria monocytogenes* in pork and poultry processing plants and products (Chasseignaux, Toquin, Ragimbeau, Salvat, Colin, & Ermel, 2001) and *Salmonella* species in pork slaughter and cutting plants (Giovannacci et al., 2001). Pathogens such as *S. aureus*, *L. monocytogenes* or *Salmonella* were detected in few environmental samples in our study. Their presences were associated to insufficient cleaning and disinfection procedures.

Surveys on foodborne diseases in Europe (Todd, 1997) have shown that when the place of contamination or mishandling could be identified, the farm was the first place where the pathogens entered the food chain (50%). Then factors contributing to outbreaks were identified as temperature abuse and particularly inadequate cooling (44%), contamination by personnel or equipment (15%), lack of hygiene in processing, preparing and handling in 10 % and cross contamination in 4%. Recontamination by pathogens through unprocessed raw materials, unclean food surfaces or personnel are fairly well recognised routes (Reij & Den Aantrekker, 2004).

In dry sausages processed on industrial scale, starter cultures are added to ensure stability and safety of the products and to enhance colour and aroma. In small-scale units, the fermentation of sausages only depend on the naturally occurring microflora (Samelis, Metaxopoulos, Vlassi, & Pappa, 1998; Talon, Leroy-Sétrin, & Fadda, 2004). This “house microflora” is supposed to be present in the environment and brought by the raw material (pork meat, fat, casings). In this study, surfaces and equipments had residual contaminations for all microbial population studied, varying from not detected to high levels of contamination. Chevallier et al. (2006) showed that spoilage (*Pseudomonas* and enterobacteria), yeasts and moulds, enterococci and LAB were practically undetected on the surfaces of a French processing unit manufacturing traditional sausages, except on block and stuffing machines where microbial levels were sometimes high. Gram-positive Catalase-positive cocci were always detected on the surfaces at level ranging from 0.5 to 4.7 log cfu/cm<sup>2</sup>. Corbière Morot-Bizot et al. (2006)



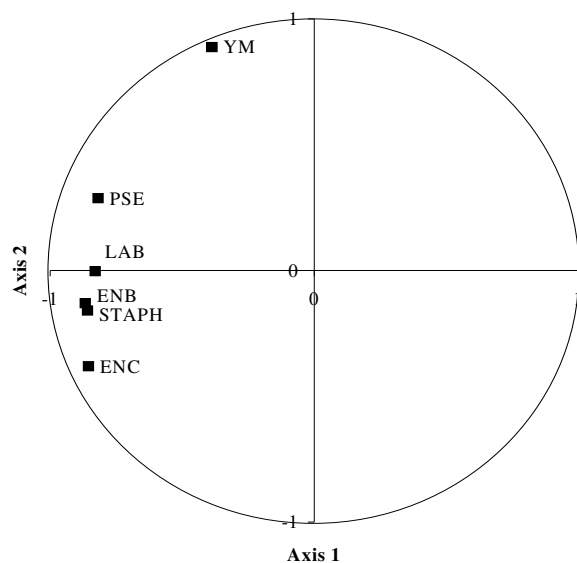
showed also that all environmental samples were colonized by Gram-positive Catalase-positive cocci with counts ranging from 2.3 to 7.0 log cfu/100 cm<sup>2</sup>.

## **5. Conclusion**

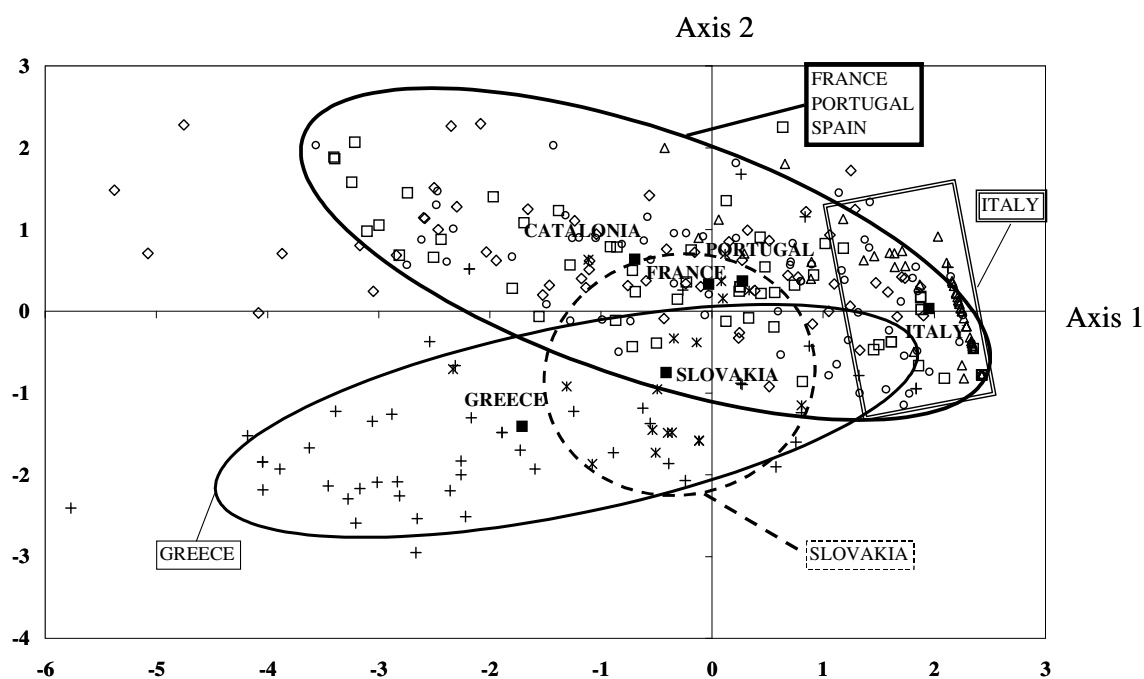
The study performed in 54 processing units originated from different Mediterranean countries and Slovakia showed that there was a great diversity of microbial populations on the environmental samples. This diversity is in term of type of microorganisms and of microbial levels for each population. This diversity is the consequence of the diversity of processing in the processing unit.

### **Acknowledgements**

This work was financially supported by EU program QLK1-CT2002-02240.



(a)



(b)

Figure 1. Principal Component Analysis (axis 1 – 62.1% and axis 2 – 17.6%) on the surface microbial data analysed in the processing units of the six countries.

(a) Scree plot, presentation of the variables. YM: Yeasts and Moulds; LAB: Lactic Acid Bacteria; STAPH: *Staphylococcus* and *Kocuria*; ENC: *Enterococcus*; ENB: *Enterobacteriaceae*; PSE *Pseudomonas*.

(b) Presentation of the samples (53 processing units x 6 surface samples)

□ France; + Greece; △ Italy; ○ Portugal; ◇ Spain; \* Slovakia; ■ Mean and name of each country

Table 1. Characteristics of the processing units (PU)

Country	Geographic area	Enquired PU	Number employees	PU selected
France	Massif Central region	108	3	F01 to F10
Greece	13 regions located in all the country	50	4.3	G01 to G10
Italy Southern	Abruzzo region	25	2.3	IS01 to IS05
Italy Northern	Emilia Romagna and Lombardia region	25	4.6	IN01 to IN05
Portugal Southern	Alentejo region	26	8.7	PS01 to PS06
Portugal Northern	Province of Tráz-os-Montes e Alto Douro	25	3	PN01 to PN05
Spain	Catalonia region	50	4.7	C01 to C10
Slovakia	All country	6	8.5	S01 to S03
Average			4.9	
		315		

Table 2: Microbial analysis

Microflora	Medium	Incubation	References and ISO
<i>Enterobacteriaceae</i>	crystal Violet neutral Red Bile Glucose agar (VRBG)	37°C - 24 h	Merck (1.05262) ISO 7402:1993
<i>Pseudomonas</i> spp.	Cetrimide-Fucidin-Cephaloridine agar (CFC)	25°C - 48 h	Oxoid (CM559, supplemented with selective supplement SR 103E, Basingstoke, UK) ISO 13720:1995
Lactic acid bacteria	Man-Rogosa-Sharp (MRS)	30°C - 48/72 h anaerobic	Merck (1.10660) ISO 15214:1998
<i>Staphylococcus</i> and <i>Kocuria</i> Enterococci	Mannitol Salt phenol red Agar (MSA) M- <i>Enterococcus</i> (ME)	30°C - 48 h 37°C - 48 h	Merck (1.05404) Merck (1.05262)
Yeasts and moulds	Yeast extract Glucose Chloramphenicol agar (YGC)	25°C - 2 days	Merck ISO13681:1995
<i>Salmonella</i>	- Semi-solid Rappaport-Vasiliadis Medium (MSRV, supplemented with MSRV selective supplement)	42°C - 24 h	Merck  Merck
<i>Staphylococcus aureus</i> and coagulase positive staphylococci	- Brilliant-green Phenol-red Lactose Sucrose agar - Baird Parker agar supplemented with Tellurite Yolk Egg (BP+TYE)	37°C - 24/48 h	Merck, BP+TYE (1.05406)  AES Laboratory, (AES 620314, AEB 184106) ISO6888-1:1999
<i>Listeria monocytogenes</i>	- Baird Parker agar supplemented with RPF (BP+RPF) <i>Listeria</i> Agar acc. to Ottaviani & Agosti (ALOA)	37°C - 48 h	AES Laboratory (520079/80) ISO11290-2:1998 ISO11290-2:1998/Amd 1:2004

Comments from Maria : For enterococci there is only a ISO for its enumeration in water  
Does references exist for ISO ?

Table 3: PCR amplification conditions

Species	Amplified fragment	Target	Primer sequence	PCR Cyclers profiles
<i>Salmonella</i>	284 bp	<i>InvA</i> (a)	139: 5'-GTGAAATTATCGCCACGTTTCGGGCAA 141: 5'-TCATCGCACCGTCAAAGGAACC	P: 94°C/1 min; DC: 30 cycles of 94°C/30 s.; A: 64°C/30 s.; E: 72°C/1 min; FI: 72°C/5 min.
<i>Listeria monocytogenes</i>	274 bp	<i>prfA</i> (b)	lip1: 5'-GATACAGAAACATCGGTTGGC lip2: 5'-GTGTAACCTTGATGCCATCAGG	P: 94°C/2 min, DC: 30 cycles 94°C/30 s., A: 55°C/30 s., E: 72°C/1 min, FI: 72°C/5 min.
<i>S. aureus</i>	108 pb	Unknown (c)	Sa442-1: 5'-AATCTTTGTCGGTACACGATATTCTTCACG Sa442-2: 5'-CGTAATGAGATTTTCAGTAGATAATACAACA	P: 96°C/3min; DC: 30 cycles of 95°C/1 s.; A: 55 C/30 s.; E: 72°C/30 s.; FI: 72°C/5 min.

(a) Rahn et al.(1992); (b) Simon et al. (1996); (c) Martineau et al. (1998).

The reaction conditions were: Preincubation (P), Denaturation Cycles (DC), Annealing (A), Extension (E) and Final Incubation (FI)

Table 4: Occurrence of pathogenic bacteria on the processing surfaces and equipments

Country	n	<i>Salmonella</i> <sup>1</sup>	<i>S. aureus</i> <sup>2</sup>	<i>L. monocytogenes</i>	
				Enumeration <sup>3</sup>	Detection PCR <sup>4</sup>
France	57	0	0	2	2
Greece	48	14	6	0	0
Italy	65	0	4	1	ND
Portugal	66	1	6	3	10
Spain	60	0	1	1	5
Slovakia	18	0	2	0	0
Total	314	15	19	7	17
%		4.8%	6.1%	2.2%	

ND, Not Determined; n, total number of environmental samples studied,

<sup>1</sup> number of positive samples after analysis of *Salmonella* on 40 cm<sup>2</sup>; <sup>2</sup> number of positive samples, detection limit for *S. aureus* 1.7 log cfu/100 cm<sup>2</sup>; <sup>3</sup> number of positive samples, detection limit for *L. monocytogenes* 1.0 log cfu/100 cm<sup>2</sup>; <sup>4</sup> sample negative in enumeration but positive by PCR after enrichment

Table 5: Multiple analysis of variance to evaluate the effect on the countries and the samplings on the microflora.

	df	YM				LAB				STAPH			
		SS	MS	F	p	SS	MS	F	p	SS	MS	F	p
Intercept	1	1256.3	1256.3	509.5	0.000	1320.8	1320.8	363.6	0.000	2261.2	2261.2	696.9	0.000
Country	5	308.4	61.7	25.0	0.000	347.2	69.4	19.1	0.000	626.0	125.2	38.6	0.000
Sampling	5	22.4	4.5	1.8	0.109	23.0	4.6	1.3	0.279	10.4	2.1	0.6	0.669
Country*Sampling	25	72.3	2.9	1.2	0.263	172.6	6.9	1.9	0.007	66.7	2.7	0.8	0.712
Error	279	687.9	2.5			1013.5	3.6			905.2	3.2		
Total	314	1121.6				1585.2				1642.7			

	df	ENC				ENB				PSE			
		SS	MS	F	p	SS	MS	F	p	SS	MS	F	p
Intercept	1	1029.0	1029.0	359.3	0.000	1117.5	1117.5	399.1	0.000	1560.1	1560.1	375.5	0.000
Country	5	647.6	129.5	45.2	0.000	423.3	84.7	30.2	0.000	393.6	78.7	18.9	0.000
Sampling	5	37.9	7.6	2.6	0.024	75.9	15.2	5.4	0.000	82.3	16.5	4.0	0.002
Country*Sampling	25	74.3	3.0	1.0	0.418	156.1	6.2	2.2	0.001	116.1	4.6	1.1	0.322
Error	279	799.0	2.9			781.1	2.8			1159.3	4.2		
Total	314	1612.0				1503.5				1774.9			

SS, sums of the squares; df, degree of freedom; MS, mean squares; F, Fisher; p, probability.

No significant effect,  $p > 0.05$ ; Significant effect,  $0.01 < p \leq 0.05$ ; Very significant effect,  $0.001 < p \leq 0.01$ ; Highly significant effect,  $p \leq 0.001$

YM, Yeasts and Moulds; LAB, Lactic Acid Bacteria; STAPH, *Staphylococcus* and *Kocuria*; ENC, *Enterococcus*; ENB, *Enterobacteriaceae*; PSE, *Pseudomonas*.

Table 6: Newman-Keuls tests to evaluate the effect of the country on the microbiota

Mean	YM	LAB	STAPH	ENC	ENB	PSE
Italy	1.7 a	0.3 b	0.3 e	0.1 c	0.3 c	0.7 b
France	3.0 b	2.4 a	2.9 a b	1.6 a b	1.6 a	2.7 a
Portugal	2.8 b	1.5 c	2.3 a	1.2 b	1.8 a b	3.0 a
Spain	3.7 d	2.7 a	3.6 b c	2.1 a	2.2 a b	3.6 a
Slovakia	1.5 a	3.4 a	4.3 c d	2.3 a	2.6 b	1.2 b
Greece	0.7 c	3.5 a	4.6 d	5.0 d	4.3 d	3.6 a

Data expressed as the mean microflora levels per countries, in logCFU/100 cm<sup>2</sup>;

Per column, two similar letters indicated that a country belonged to a similar group.

YM: Yeasts and Moulds; LAB: Lactic Acid Bacteria; STAPH: *Staphylococcus* and *Kocuria*; ENC: *Enterococcus*; ENB: *Enterobacteriaceae*;

PSE *Pseudomonas*.



Table 7: Newman-Keuls tests to evaluate the effect of the samplings on the microbiotia

Mean	YM		LAB		STAPH		ENC		ENB		PSE	
Cold room	1.8	b	1.6	a	2.5	a	1.4	a	1.0	b	1.8	a
Mixing machines	2.0	a b	1.7	a	2.5	a	1.5	a	1.4	b	2.2	a
Stuffing machines	2.4	a b	2.1	a	2.6	a	1.7	a	2.2	a	2.4	a b
Mincing machines	2.4	a b	2.4	a	3.1	a	2.3	a	2.2	a	2.7	a b
Knives	2.8	a	2.4	a	2.9	a	2.2	a	2.5	a	3.3	b
Tables	2.8	a	2.4	a	3.0	a	2.2	a	2.6	a	3.3	b

Data expressed as the mean microflora levels per countries, in logCFU/100 cm<sup>2</sup>.

Per column, two similar letters indicated that an environmental sample belonged to a similar group.

YM: Yeasts and Moulds; LAB: Lactic Acid Bacteria; STAPH: *Staphylococcus* and *Kocuria*; ENC: *Enterococcus*; ENB: *Enterobacteriaceae*; PSE *Pseudomonas*.

Range of microbial association (log cfu/100 cm<sup>2</sup>) on the processing surfaces and equipments for each country

Country	Microorganisms	Tables		Knives		Cold room		Mincing machines		Mixing machines		Stuffing machines	
		Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
France n=10	YM	<0.7	6.8	2.7	6.7	<0.7	6.7	1.5	4.7	<0.7	5.4	<0.7	6.7
	LAB	<1.7	7.1	1.7	5.6	<1.7	5.0	<1.7	5.5	<1.7	5.8	<1.7	5.4
	STAPH	<1.7	6.9	<1.7	5.3	<1.7	4.8	1.7	5.5	<1.7	5.3	<1.7	5.7
	ENC	<0.7	3.1	<0.7	5.0	<0.7	4.7	<0.7	3.4	<0.7	4.0	<0.7	3.9
	ENT	<0.7	6.4	<0.7	6.0	<0.7	1.7	<0.7	3.1	<0.7	<0.7	<0.7	6.1
	PSE	<0.7	7.1	2.7	7.3	<0.7	3.9	<0.7	5.3	<0.7	3.5	<0.7	7.1
Greece n=10	YM	3.4	5.5	<0.7	7.8	<0.7	8.2	2.9	7.2	4.2	6.4	<0.7	5.9
	LAB	<1.7	6.0	<1.7	7.3	<1.7	7.0	<1.7	6.8	<1.7	8.2	2.8	6.1
	STAPH	<1.7	5.5	<1.7	6.2	<1.7	6.2	<1.7	7.3	4.7	6.7	<1.7	5.3
	ENC	<0.7	5.9	<0.7	6.5	<0.7	5.9	<0.7	7.7	<1.7	6.9	2.2	3.0
	ENT	2.0	6.8	<0.7	8.6	<0.7	7.1	<0.7	7.0	3.9	6.9	2.3	7.0
	PSE	3.0	8.7	<0.7	9.4	<0.7	6.2	<0.7	7.8	3.9	7.2	<0.7	8.8
Italy n=10	YM	<0.7	4.2	<1.3	5.3	<0.7	3.0	<0.7	3.7	<0.7	3.3	0.9	3.1
	LAB	<1.7	4.2	<1.7	3.2	<1.7	2.3	<1.7	2.3	<1.7	1.9	<1.7	1.9
	STAPH	<1.7	3.9	<1.7	2.0	<1.7	2.6	<1.7	2.5	<1.7	1.8	<1.7	2.6
	ENC	<0.7	2.5	<1.3	1.3	<0.7	<0.7	<0.7	<0.7	<0.7	<0.7	<0.7	<0.7
	ENT	<0.7	2.9	<1.3	2.4	<0.7	<0.7	<0.7	2.30	<0.7	<0.7	<0.7	<0.7
	PSE	<0.7	5.0	<1.3	5.1	<0.7	1.9	<0.7	4.7	<0.7	1.9	<0.7	0.8

Portugal	YM	<0.7	7.2	1.5	5.4	<0.7	5.1	0.7	5.1	<0.7	4.6	0.7	3.2
n=10	LAB	<0.7	4.8	<0.7	4.7	<0.7	3.5	<0.7	4.7	<0.7	5.0	<0.7	5.0
	STAPH	<0.7	5.2	<0.7	5.9	<0.7	3.4	<0.7	5.2	<0.7	4.5	<0.7	3.7
	ENC	<0.7	3.7	<0.7	5.2	<0.7	1.6	<0.7	4.2	<0.7	3.0	<0.7	2.8
	ENT	<0.7	6.4	<0.7	4.3	<0.7	3.3	<0.7	5.5	<0.7	3.6	<0.7	4.3
	PSE	<0.7	7.2	<0.7	7.6	<0.7	7.1	<0.7	6.2	<0.7	6.8	<0.7	6.2
Spain	YM	1.2	7.1	1.8	6.8	<0.7	4.8	2.7	7.0	1.2	4.5	2.5	8.1
n=10	LAB	<1.7	8.3	<1.7	5.00	<1.7	3.3	2.4	8.6	<1.7	4.2	<1.7	8.4
	STAPH	<1.7	5.7	<2.0	5.8	<1.7	8.2	3.2	5.9	0.7	4.8	<1.7	7.0
	ENC	<0.7	4.7	<0.7	4.4	<0.7	2.4	1.3	6.1	<0.7	4.1	<0.7	6.1
	ENT	<0.7	5.7	<0.7	3.2	<0.7	2.2	<0.7	8.3	<0.7	3.5	<0.7	7.9
	PSE	<0.7	8.0	<0.7	8.0	<0.7	4.0	2.0	8.0	<0.7	4.2	<0.7	8.9
Slovakia	YM	<0.7	3.6	<0.7	2.8	<0.7	4.9	<0.7	4.2	<0.7	4.1	<0.7	4.7
n=5	LAB	<0.7	5.4	2.6	3.6	3.7	4.5	3.5	4.7	<0.7	4.3	2.5	4.6
	STAPH	2.9	6.7	2.4	4.3	3.8	5.5	3.7	4.7	3.5	4.7	3.5	6.6
	ENC	2.4	4.5	<0.7	4.1	<0.7	2.7	2.4	4.5	<0.7	3.3	<0.7	4.1
	ENT	2.8	3.7	2.1	3.4	<0.7	3.3	1.7	3.4	2.1	4.4	2.1	2.4
	PSE	2.5	4.1	<0.7	3.7	<0.7	1.1	<0.7	4.5	<0.7	2.4	<0.7	<0.7

Min, minimum; Max, maximum. YM, Yeasts and Moulds; LAB, Lactic Acid Bacteria; STAPH, *Staphylococcus* and *Kocuria*; ENC, *Enterococcus*; ENB, *Enterobacteriaceae*; PSE, *Pseudomonas*.

## Reference

1. Aslam, M., Greer, G. G., Nattress, F. M., Gill, C. O., & McMullen, L. M. (2004). Genotypic analysis of *Escherichia coli* recovered from product and equipment at a beef-packing plant. *Journal of Applied Microbiology*, 97, 78-86.
2. Aymerich, T., Martín, B., Garriga, M., & Hugas, M. (2003). Microbial quality and direct PCR identification of lactic acid bacteria and nonpathogenic Staphylococci from artisanal low-acid sausages. *Applied and Environmental Microbiology*, 69, 4583-4594.
3. Chasseignaux, E., Toquin, M. T., Ragimbeau, C., Salvat, G., Colin, P., & Ermel, G. (2001). Molecular epidemiology of *Listeria monocytogenes* isolates collected from the environment, raw meat and raw products in two poultry- and pork-processing plants. *Journal of Applied Microbiology*, 91, 888-899.
4. 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.
5. Comi, G., Urso, R., Iacumin, L., Rantsiou, K., Cattaneo, P., Cantoni, C., & Cocolin, L. (2005). Characterisation of naturally fermented sausages produced in the North East of Italy. *Meat Science*, 69, 381-392.
6. Conter, M., Zanardi, E., Ghidini, S., Pedrelli, P., Rason, J., & Chizzolini, R. (2005). Indagine sui produttori artigianali di salumi: valutazioni socioeconomiche e tecnologiche. *Industrie Alimentari*, 446,
7. Corbiere Morot-Bizot, S., Leroy, S., & Talon, R. (2006). Staphylococcal community of a small unit manufacturing traditional dry fermented sausages. *International Journal of Food Microbiology*, 108, 210-217.
8. Council Regulation (EC) 509 (2006). Council Regulation (EC) 509/2006 of 20 March 2006 on agricultural products and foodstuffs as traditional specialities guaranteed. *Official Journal of European Communities*, No. L 093, 111.
9. Council Regulation (EC) 510 (2006). Council Regulation (EC) 510/2006 of 20 March 2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs. *Official Journal of European Communities*, No. L 093, 1225.
10. Gill, C. O. & McGinnis, J. C. (2004). Microbial conditions of air knives before and after maintenance at a beef packing plant. *Meat Science*, 68, 333-337.
11. Giovannacci, I., Queguiner, S., Ragimbeau, C., Salvat, G., Vendevre, J. L., Carlier, V., & Ermel, G. (2001). Tracing of *Salmonella* spp. in two pork slaughter and cutting plants using serotyping and macrorestriction genotyping. *Journal of Applied Microbiology*, 90, 131-147.
12. Kuznesof, S., Tregear, A., & Moxey, A. (1997). Regional foods: a consumer perspective. *British Food Journal*, 99, 199-206.
13. Lebert, I., Leroy, S., Giammarinaro, P., Lebert, A., Chacornac, J. P., Bover-Cid, S.,

- Vidal, M., & Talon, R. (2007). Diversity of micro-organisms in environments and dry fermented sausages of French traditional small units. *Meat Science*, *in press*,
14. Mauriello, G., Casaburi, A., Blaiotta, G., & Villani, F. (2004). Isolation and technological properties of coagulase negative staphylococci from fermented sausages of Southern Italy. *Meat Science*, *67*, 149-158.
  15. Papamanoli, E., Tzanetakis, N., Litopoulou-Tzanetaki, E., & Kotzekidou, P. (2003). Characterization of lactic acid bacteria isolated from a Greek dry-fermented sausage in respect of their technological and probiotic properties. *Meat Science*, *65*, 859-867.
  16. Rahn, K., De Grandis, S. A., Clarke, R. C., McEwen, S. A., Galan, J. E., Ginocchio, C., Curtiss, R. 3rd, & Gyles, C. L. (1992). Amplification of an *invA* gene sequence of *Salmonella typhimurium* by polymerase chain reaction as a specific method of detection of *Salmonella*. *Molecular Cell Probes*, *6*, 271-279.
  17. Rantsiou, K., Urso, R., Iacumin, L., Cantoni, C., Cattaneo, P., Comi, G., & Cocolin, L. (2005). Culture-dependent and -independent methods to investigate the microbial ecology of Italian fermented sausages. *Applied and Environmental Microbiology*, *71*, 1977-1986.
  18. Rason, J., Lebecque, A., Leger, L., & Dufour, E. (2004). Diversité des ateliers du Massif Central. *Viandes Et Produits Carnés*, *24*, 31-41.
  19. Reij, M. W. & Den Aantrekker, E. D. (2004). Recontamination as a source of pathogens in processed foods. *International Journal of Food Microbiology*, *91*, 1-11.
  20. Röhr, A., Lüddecke, K., Drusch, S., Müller, M. J., & Alvensleben, R. V. (2005). Food quality and safety-consumer perception and public health concern. *Food Control*, *16*, 649-655.
  21. Samelis, J., Metaxopoulos, J., Vlassi, M., & Pappa, A. (1998). Stability and safety of traditional Greek salami - a microbiological ecology study. *International Journal of Food Microbiology*, *44*, 69-82.
  22. Simon, C. M., Gray, D. I., & Cook, N. (1996). DNA extraction and PCR methods for the detection of *Listeria monocytogenes* in cold-smoked Salmon. *Applied and Environmental Microbiology*, *62*, 822-824.
  23. Talon, R., Leroy-Sétrin, S. & Fadda, S. (2004). Chapter 23: Dry Fermented sausages. In: Y. H. Hui, L. Meunier-Goddik, A. S. Hansen, J. Josephsen, W.-K. Nip, P. S. Stanfield, & F. Toldrá (Eds), *Handbook of Food and Beverage Fermentation Technology*, 1st Ed., (pp. 397-416). New York: Marcel Dekker Inc.
  24. Todd, E. C. D. (1997). Epidemiology of foodborne diseases: a worldwide review. In: World Health Organization (Ed.), *World Health statistics quartely*, (50) (pp. 30-50). Geneva: World Health Organization.
  25. Wilcock, A., Pun, M., Khanona, J., & Aung, M. (2004). Consumers attitudes, knowledge and behaviour: a review of food safety issues. *Trends in Food Science and Technology*, *15*, 56-66.

