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Chapter 6

MICROBIAL ECOSYSTEM OF TRADITIONAL DRY FERMENTED SAUSAGES IN MEDITERRANEAN COUNTRIES AND SLOVAKIA

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ABSTRACT

This study highlighted the wide diversity of the processes for the manufacturing of traditional dry sausages in the Mediterranean countries (Spain, Italy, France, Greece and Portugal) and East-Central Europe (Slovakia). In particular, the temperature range of the "fermentation" step was broad, from low temperature 2°C to high 26°C together with variable length (1-8 days). Also a wide range both in temperature (2-25°C) and length (5-90 days) was noticed for the ripening step.

Statistical analyses revealed that Gram-positive catalase-positive cocci (GCC+), yeasts/moulds and *Enterobacteriaceae* discriminated the ripened sausages according to the geographical origins while Lactic Acid Bacteria (LAB) constituted the dominant bacteria of the ripened products in all the countries. LAB counts, which increased during the fermentation step and a_w which decreases during ripening, discriminated the three manufacturing steps (batter, fermented, ripened).

In general, traditional dry fermented sausages did not present health risks, although the presence of some pathogens and spoilage microbiota in some sausages highlighted the importance of maintaining sound hygienic procedures.

Keywords: Traditional dry fermented sausages; Technological microbiota; Spoilage microbiota; Pathogen; Process.

1. INTRODUCTION

The demand for traditional food production has increased all over Europe since the 1980's (Wilcock et al. 2004; Röhr et al. 2005). This demand is linked to the consumer's desire for natural, authentic and safe products. Although Europe is the major producer and consumer of these products, a wide variety of traditional dry fermented sausages can be found worldwide (Campbell-Platt 1995). National or local differences arise in the choice of raw materials, degrees of mincing, sizes, recipes, fermentation and ripening conditions and durations. The wide variety of sensory characteristics of these fermented meat products derives from the manufacturing practices used in the various countries and regions. Traditional products are usually naturally fermented (Aymerich et al. 2003; Comi et al. 2005; Drosinos et al. 2005; Chevallier et al. 2006). Since no starter cultures are added, the microbiota that grows is related to the diversity of the formulations and to the fermentation and ripening practices. The knowledge and the control of the in-house microbiota during the processing are essential in terms of microbiological quality, sensory characteristics and safety of traditional dry fermented sausages. This microbiota is composed of technological microorganisms useful for fermentation, spoilage microorganisms that can cause negative changes in the sensory properties of the final product due to their metabolic activity, and may also include pathogenic microorganisms. Biogenic amines are another safety concern in fermented meat products because of their potential toxicological effects and from the hygienic and technological point of view (Latorre-Moratalla et al. 2008; Latorre-Moratalla et al. 2010).

This study presents a survey of the microbial ecosystem of traditional dry fermented sausages produced by 54 processing units located in five Mediterranean countries (Spain, Italy, France, Greece and Portugal) and East-Central Europe (Slovakia). The objective was to

assess the safety of the sausages and to find which parameters discriminated the sausages at the three steps of the process and which ones discriminated the ripened sausages according to the geographical origin.

2. MATERIALS AND METHODS

2.1. Processing Units and Sausage Manufacturing

Fifty-four traditional processing units (PUs) were selected according to the method developed by Rason et al. (2006) from a total of 314 PUs studied within the framework of the European project "Tradisausage" (Talon et al. 2007). These PUs were located in European Mediterranean countries and Slovakia (Table 1). The average sausage production per country was comprised between 1.1 and 33.7 tones per year. Most of the sausages were manufactured with lean pork and pork fat, stuffed into natural casings, with weights varying from 150 g-350 g. Different ratios of lean meat and fat were used, ranging from 90/10, 80/20 to 50/50. Glucides were generally added in low amounts (< 8 gKg^{-1}), and starters were occasionally added (Table 1). NaCl was added to all sausages at variable levels, up to a maximum of 30 g Kg^{-1}, whereas nitrite and/or nitrate salts were not added or could be added as a mixture of curing salts (sodium chloride and nitrate), up to a maximum level of 0.6 g Kg^{-1}.

The stuffed batters were processed in two consecutive and separate stages, referred to as fermentation and ripening, respectively. The fermentation was distinguished by low or high temperatures. Processing conditions during fermentation and ripening are presented in Table 1. Certain sausages from Portugal, Greece and Slovakia were smoked.

2.2. Sampling and Sample Analysis

Three sausage samples (the batter just stuffed, the product at the end of the fermentation period and the product at the end of ripening) were analysed for each processing unit. Casing was removed before analysis. Twenty five g of product were aseptically transferred to 225 ml of sterile buffered peptone water solution (BPW, AES Laboratory) and homogenised with a stomacher. For *Listeria monocytogenes* enumeration, 10 g of product were diluted in 20 ml of the same diluent. Serial dilutions in BPW were performed and duplicate 1 ml or 0.1 ml samples of appropriate dilutions were poured or spread on different media.

Enumeration of *Pseudomonas*, yeasts and moulds, lactic acid bacteria (LAB), Gram-positive catalase-positive cocci (GCC+), *Enterobacteriaceae*, *Enterococcus*, *Staphylococcus aureus* and other coagulase positive staphylococci, and *L. monocytogenes* were performed according to the methods given in Table 2 as described by Talon et al. (2007). Occurrence of *Salmonella* and Shiga toxin-producing *Escherichia coli* (STEC) was performed as described in Table 2. The STEC were detected in ripened sausages using specific PCR analysis as described (Schmidt et al. 1994; Karch and Meyer 1989) according to the reaction conditions described by Lebert et al. (2007).

Table 1. Characteristics of the processing units (PU) and of the processes

Country	PUs surveyed	PU name[1]	PUs using Starters	Glucides (g/kg)	Smoking	Fermentation Low temperature (<18°C) T[2] range (°C)	Time range (days)	PUs	High temperature (≥18°C) T[2] range (°C)	Time range (days)	PUs	Ripening T[2] range (°C)	Time range (days)
France	10	F01 to F10	F10	0 - 8	None	10 - 16	5 - 8	F01 to F04, F06, F09	18 - 22	2 - 3	F05, F07, F08, F10	8 - 14	31 - 82
Spain	10	C01 to C10	C03, C10	0 - 33	None	2 - 17	<1-5	C02 to C10	24	2	C01	10 – 18	15 - 60
Italy	10	IS01 to IS05 IN01 to IN05	None	0 – 3	None	4 - 16	1 - 10	IS02, IS05, IN02, IN03, IN05	18 - 26	1 - 8	IS01, IS03, IS04, IN01, IN04	6 – 22	15 - 90
Portugal	11	PS01 to PS06 PN01 to PN05	None	None	All	2 - 12	1 - 3	PS01 to PS06, PN01 to PN05			None	2 – 21	5 - 45
Greece	10	G01 to G10	G01	0 - 3	G01, G09, G10	12 - 15	1 - 3	G03, G05, G06, G07, G09, G10	18 - 24	3 - 7	G01, G02, G04, G08	12 - 17	14 - 60
Slovakia	3	S01 to S03	S02	0 - 6	All	15 - 16	5 - 12	S01 to S03			None	15 – 25	12 - 21

[1] F: France, C: Spain, IS: Italy South, IN: Italy North; PS: Portugal South, PN: Portugal North, G: Greece, S: Slovakia.

[2] Temperature.

Table 2. Microbial Analysis

Microbiota	Medium	Incubation	References and ISO
Enterobacteriaceae	Crystal Violet Neutral Red Bile Glucose agar (VRBG)	37°C - 24 h	Merck, Darmstad, Germany ISO 7402:1993
Pseudomonas spp.	Cetrimide-Fucidin-Cephaloridine agar (CFC)	25°C - 48 h	Oxoid (Basingstoke, United Kingdom) ISO 13720:1995
Lactic acid bacteria	Man-Rogosa-Sharpe (MRS)	30°C - 48/72 h anaerobic	Merck, Darmstad, Germany ISO 15214:1998
Staphylococcus and *Kocuria*	Mannitol Salt Phenol Red agar (MSA)	30°C - 48 h	Merck, Darmstad, Germany
Enterococcus	M-*Enterococcus* (ME)	37°C - 48 h	Merck, Darmstad, Germany
Yeasts and moulds	Yeast Extract Glucose Chloramphenicol agar (YGC)	25°C - 48 h	Merck, Darmstad, Germany ISO13681:1995
Salmonella (presence/absence) BPW enrichment 24h-48h 37°C	- Semi-solid Rappaport-Vasiliadis Medium (MSRV, supplemented with MSRV selective supplement)	42°C - 24 h 37°C - 48 h	Merck, Darmstad, Germany Merck, Darmstad, Germany
	- Brillant-green Phenol-red Lactose Sucrose agar		
Staphylococcus aureus	- Baird Parker agar supplemented with Egg Yolk, Tellurite Emulsion (BP+EYT)	37°C - 24/48 h 37ºC - 48 h	Merck, Darmstad, Germany AES Laboratory, Combourg, France ISO6888-1:1999
	- Baird Parker agar supplemented with Rabbit Plasma Fibrinogen (BP+RPF)		
Listeria monocytogenes	*Listeria* Agar acc. to Ottaviani & Agosti (ALOA)	37°C - 48 h	AES Laboratory, Combourg, France ISO11290-2:1998, ISO11290-2:1998/Amd 1:2004
Shiga Toxin *Escherichia coli* (presence/absence) PCR identification	Tryptone Soja Broth enrichment with bile extract (1.5 g l^{-1}) and novobiocine (20 mg l^{-1},	24h 37°C	Becton Dickinson, Le Pont de Claix, France Sigma Aldrich, L'Isle d'Abeau Chesnes, France Merck, Darmstad, Germany

2.3. Statistical Analysis

Two forward stepwise factorial discriminant analyses (FDA) were carried out considering as variables (i) the six microbial populations of batter, fermented and ripened products

(present study) and the physico-chemical data (pH, a_w) previously published (Latorre-Moratalla et al., 2008); (ii) the six microbial populations of the 54 final products. The discriminant function analysis was performed with the discriminant analysis routine by Statistica software (Statistica version 6.1, Statsoft inc., Maisons-Alfort, France).

3. RESULTS AND DISCUSSION

This work studied the pathogenic, spoilage and technological microbiota of 162 meat products during the manufacturing process (batters, fermented and ripened sausages) in 54 traditional processing units in five Mediterranean countries and in Slovakia.

3.1. Pathogenic Microbiota

The occurrence of pathogenic bacteria in the traditional fermented sausages studied was sporadic. STEC was not detected in any of the ripened products studied. *Salmonella* which is generally not detected in naturally fermented sausages (Aymerich et al. 2003; Comi et al. 2005; Drosinos et al. 2005; Rantsiou et al. 2005) was found in three Italian sausages both in the fermentation step and in the final product. Thus these ripened sausages did not fulfill the microbiological criteria of the Commission Regulation nº 2073/2005 (EC 2005) amended by nº 1441/2007 (EC 2007). *Salmonella* was also detected in two Spanish and one Greek products at the beginning of the process but not detected in the final products.

The percentage of samples studied contaminated by *S. aureus* decreased throughout the process from 17% in the batters to 9% in the ripened products. This contamination was noticed in sausages of five countries out of the six studied. According to the Health Protection Agency guidelines (http://www.hpa.org.uk/), *S. aureus* counts were slightly higher (3 and 3.4 log cfu/g) than the maximum tolerable limit (2.7 log cfu/g) in two ripened products. Variable results have been reported in the literature. In Salame Milano, *S. aureus* decreased during ripening until it was undetectable (Rebecchi et al. 1998), while in other Italian sausages and in French ones, *S. aureus* was still present at the end of the ripening (Blaiotta et al. 2004; Chevallier et al. 2006).

The number of *L. monocytogenes*–positive samples decreased during the process from 17% in the batters to 9 % in the final products. Only one ripened French sausage was enumerated at 2.9 log cfu/g, level higher to the established criteria for ready-to-eat products 2.0 log (cfu/g), during shelf-life, EC (2005). *L. monocytogenes* was generally not detected in ripened products (Samelis et al. 1998; Drosinos et al. 2005) or at any stage of the process (Rebecchi et al. 1998). Sporadic cases of contamination by *L. monocytogenes* at low levels were reported in Spanish sausages (Aymerich et al. 2003).

3.2. Diversity of the Products Along the Process

This study highlighted the diversity of the various processes applied in the PUs of the six countries studied. During the first step of production, known as the fermentation step, 74% of

the PUs fermentation takes place at temperatures below 18°C with a broad range (2 to 17°C), while 26% of the PUs used temperatures between 18 and 26°C (Table 1). During ripening, temperatures varied from 2 to 25°C, and ripening time varied from 5 to 60 days. The observed diversity was high both for sausages manufactured within the same country and for sausages manufactured in different countries, highlighting the high diversity of the products obtained. These results revealed a higher variability in the processing conditions than previously reported. During ripening, temperatures in Mediterranean countries usually ranged from 10 to 14°C, as reported for France (Chevallier et al. 2006), Italy (Coppola et al. 2000; Comi et al. 2005; Rantsiou et al. 2005) and Greece (Papamanoli et al. 2003). When higher drying temperatures were observed, they generally corresponded to short ripening periods, from one to three weeks (Drosinos et al. 2005).

In spite of process diversity, certain similarities could be observed in the evolution of the products. A Factorial Discriminant Analysis (FDA) was performed to find the main variables (microbiota and/or physicochemical properties) that discriminate the sausages at the three steps of the manufacturing (batter, fermented and ripened) (Table 2). The FDA showed that only two variables, a_w and LAB discriminated the samples. The classification matrix indicates that 76.7% of the samples were properly classified with 83% of the batter samples, 72% of the fermented products and 75% of the ripened sausages (Table 3). The five sausages manufactured with starter cultures (F10, C03, C10, G01, S02) were correctly classified.

Table 3. Classification matrix from the discriminant function analysis

Step of process	Samples[1]	% of correct classification[2]	Predicted classification group		
			"Batter"	"Fermented product"	"Ripened product"
Batter	53	83.0	44[3]	9[4]	0
Fermented product	53	71.7	10[5]	38[3]	5[6]
Ripened product	53	75.5	2[7]	11[8]	40[3]
Total	159	76.7	56	58	45

[1] 53 samples among 54 were considered because of missing data from one PU.

[2] Percentage of samples properly classified by the classification function.

[3] Number of products properly classified (on the diagonal of the matrix).

From [4] to [8]: Number of products misclassified in a predicted group.

[4] Misclassified products from PUs: IS01, G02, G04, G06, G07, G08, G09, G10, S01.

[5] Misclassified products from PUs: F01, F07, F08, C04, IS01, IS02, IS03, IS04, IN04, PS01.

[6] Misclassified products from PUs: C05, C06, PS03, PS04, PS05.

[7] Misclassified products from PU: C04, PS02.

[8] Misclassified products from PUs: IN01, PS01, PS06, PN05, G02, G04, G05, G06, G08, G09, S03.

F: France, C: Spain, IS: Italy South, IN: Italy North; PS: Portugal South, PN: Portugal North, G: Greece, S: Slovakia

The predicted batter group contained 56 samples composed of 44 batters, 10 fermented and 2 ripened products (Table 3). It was characterised by a high a_w and a low count of LAB (Table 4). Among the misclassified samples, three were from the Spanish PU C04 (batter, fermented and ripened products) that had typical physico-chemical and microbiological characteristics of a batter. These sausages, named "sumaia", have indeed particular

characteristics: slightly-fermented, small size sausage (low calibre) and only two weeks of ripening at 15ºC and 74-84% RH. For the other nine fermented products with batter properties, LAB levels were low and no growth occurred because fermentation took place at low temperatures (below 11°C) or/and in the absence of sugar as an ingredient.

Table 4. Microbial and physico-chemical characteristics of the predicted groups (Table 3)

Predicted group	n[1]		a_w *	LAB[3] *	PSE[4]	ENC[5]	GCC[6]	ENB[7]	YM[8]	pH
Batter	56	Mean[2]	0.963	4.5	5.0	3.1	4.0	3.5	3.9	5.8
		SD[2]	0.014	1.1	1.6	1.3	1.6	1.2	1.3	0.3
Fermented product	58	Mean	0.951	7.7	4.6	4.5	4.8	4.1	4.6	5.3
		SD	0.018	1.1	2.0	1.7	2.0	2.5	1.6	0.4
Ripened product	45	Mean	0.868	8.0	3.3	4.2	5.3	2.8	4.7	5.5
		SD	0.039	1.0	2.0	1.8	1.9	2.5	1.4	0.5

[1] Number of products in each predicted group.

[2] Mean and standard deviation of pH, a_w, microflora (log cfu/g) calculated for each predicted group.

[3] Lactic Acid Bacteria; [4] *Pseudomonas*; [5] *Enterococcus*; [6] Gram-positive catalase-positive cocci ; [7] *Enterobacteriaceae*; [8] Yeasts and Moulds.

* Significant variables from the discriminant function analysis.

pH and a_w raw values are given in Latorre-Moratalla et al. (2008).

The predicted fermented product group had 58 samples with 38 fermented products, 9 batters and 11 ripened products (Table 3). Compared with the former group, it was characterised by an increase in LAB counts and a slight decrease in a_w (Table 4). The nine batters, mainly from Greece, had the characteristics of a fermented product primarily because they had an initial LAB level similar to the one of a fermented product. Since no starter culture was added, which could have explained the high initial counts, it could be attributed to the high levels of endogenous LAB from the raw meat and the processing environment (Gounadaki et al. 2008), together with the low initial pH of the batters. The eleven ripened products that had high a_w similar to those of a fermented product were from Greece (6) and Portugal (3) (Tables 3, 4). This could be attributed to short ripening period which led to little dehydration.

The predicted ripened product group had 45 samples composed of 5 fermented and 40 ripened products (Table 3). Compared to the previous groups, it was characterised by a low a_w (Table 4). The five misclassified fermented products, three from Portugal and two from Spain, were characterised by low a_w and high LAB levels. For Portuguese sausages, this could be explained by high levels of LAB from both the raw meat and the processing environment, including knives, tables and stuffing machines (Talon et al. 2007). The small diameter of the Spanish and of two of the Portuguese sausages would explain the low a_w.

3.3. Diversity of the Final Products

A Factorial Discriminant Analysis (FDA) was performed to find the main microbiota that discriminate the final sausages manufactured in the six countries. The FDA showed that three variables, *Enterobacteriaceae*, GCC+ and yeasts/moulds, discriminated the samples. The classification matrix showed that 61.1% of the products were properly classified (Table 5).

Table 5. Classification matrix from the discriminant function analysis

Country	Samples[1]	% of correct classification[2]	Predicted classification					
			France	Spain	Italy	Portugal	Greece	Slovakia
France	10	70.0	7[3]	0	0	1	1	1
Spain	10	50.0	3	5[3]	2	0	0	0
Italy	10	60.0	0	2	6[3]	1	1	0
Portugal	11	54.5	0	1	1	6[3]	2	1
Greece	10	70.0	0	0	2	0	7[3]	1
Slovakia	3	66.7	0	0	0	1	0	2[3]
Total	54	61.1	10	8	11	9	11	5

[1] Number of ripened sausages studied.
[2] Percentage of samples properly classified by the classification function.
[3] Number of products properly classified.

France and Greece had the highest percentage (70%) of sausages properly classified while Portugal (54%) and Spain (50%) had the lowest ones. The France predicted group, composed for the most part (70%) of French sausages, was mainly characterised by high level of GCC+ and yeasts/moulds (Table 6). The Spain predicted group (with 62.5% of Spanish sausages) was characterised by high level of CGC+, yeasts/moulds and low level of *Enterobacteriaceae*. The predicted Greece (63.6% of Greek sausages) and Portugal (66.6% of Portuguese sausages) groups were mainly discriminated for their higher level of *Enterobacteriaceae*. The predicted Italy group had the lowest level of GCC+ while the Slovakia predicted group had the lowest level of yeasts/moulds (Table 6).

Despite not all the sausages could be gathered according to their original country, three variables, GCC+, yeasts/moulds and *Enterobacteriaceae* discriminated the ripened sausages according to the geographical origin. GCC+ constituted the second dominant microbiota after LAB in French, Spanish and Portuguese predicted groups while GCC+ level was low in the Greek, Italian and Slovakian groups. LAB constituted whatever the processing countries the dominant microbiota and did not discriminate the sausages. It is well established that LAB represented the dominant microbiota in traditional sausages, and the species most commonly identified are *Lactobacillus sakei*, *Lactobacillus curvatus* and *Lactobacillus plantarum* (Ammor et al. 2005; Rantsiou et al. 2005 ; Aymerich et al. 2006 ; Lebert et al. 2007b). Variable levels of GCC+ were already observed in European products by many authors, with level inferior to 5.0 log CFU/g for some Greek and Italian sausages, often in relation with low pH (Rebecchi et al. 1998; Samelis et al. 1998; Cocolin et al. 2001; Aymerich et al. 2003; Mauriello et al. 2004; Comi et al. 2005; Lebert et al. 2007a).

Table 6. Microbial characteristics of the predicted groups (Table 5)

Predicted group	n[1]		ENB[3]*	GCC+[4]*	YM[5]*	LAB[6]	PSE[7]	ENC[8]
France	10	Mean[2]	1.8	6.5	5.3	8.1	4.0	4.7
F01, F02, F03, F04, F06, F07, F08, C05, C07, C09		SD[2]	1.4	0.8	0.6	0.7	0.7	1.3
Spain	8	Mean[2]	0.9	5.9	5.5	7.8	1.4	4.1
C02, C03, C06, C08, C10, IN03, IN04, PN03		SD[2]	1.0	1.4	0.6	0.8	1.5	1.2
Greece	11	Mean[2]	6.6	4.5	5.2	8.5	6.1	5.3
G01, G04, G05, G06, G08, G09, G10, F05, IN05, PS03, PS06		SD[2]	2.2	2.5	1.5	0.8	1.9	2.2
Italy	11	Mean[2]	1.6	3.3	4.8	6.5	3.1	2.5
IS02, IS03, IS04, ISO5, IN01, IN02, C01, C04, PN04, G02, G07		SD[2]	1.4	2.0	1.2	1.1	1.7	1.1
Portugal	9	Mean[2]	4.9	5.8	5.0	7.2	3.5	3.8
PS02, PS04, PS05, PN01, PN02, PN05, F09, IS01, S03		SD[2]	1.4	0.9	1.1	1.4	2.1	1.2
Slovakia	5	Mean[2]	0.8	4.9	1.0	8.8	2.0	5.8
S01, S02, F10, PS01, G03		SD[2]	1.3	2.2	1.0	1.6	1.1	1.5

[1] Number of products in each predicted group.

[2] Mean and standard deviation of microflora (log cfu/g) for each predicted group.

[3] *Enterobacteriaceae*; [4] Gram-positive catalase-positive cocci; [5] Yeasts and Moulds; [6] Lactic Acid Bacteria; [7] *Pseudomonas*; [8] *Enterococcus.*

* Significant variables from the discriminant function analysis.

F: France, C: Spain, IS: Italy South, IN: Italy North; PS: Portugal South, PN: Portugal North, G: Greece, S: Slovakia

Staphylococcus xylosus is the most common species identified in traditional sausages and *Staphylococcus saprophyticus* is often the second one and could be dominant in some Greek and Italian sausages (Mauriello et al. 2004; Drosinos et al. 2005; Simonová et al. 2006; Lauková et al. 2010). In Italian and French products, *Staphylococcus equorum* and *Staphylococcus succinus* were isolated with *S. equorum* being largely dominant (Mauriello et al. 2004; Leroy et al. 2010).Yeasts and moulds constituted an important part of the microbiota except for the group of Slovakia. This group is mainly composed of sausages with large diameter or/and smoked. Variable growth of yeasts and moulds has been reported by some authors. Some observed slow growth during the ripening period (Drosinos et al. 2005), others reported a decrease in the population (Comi et al. 2005) and others did not detect them at the end of ripening (Rebecchi et al. 1998). *Enterobacteriaceae* discriminated the final sausages with Greek (6.6 log cfu/g) and Portuguese (4.6 log cfu/g) the most contaminated ones. For most of these sausages, the batters were highly contaminated and the process did not reach the conditions that decrease this population, indeed Greek sausages were characterised by high a_w (0.92-0.95), while the other sausages had intermediate a_w (0.90) combined with mild pH (5.6-

5.8). Considering the spoilage bacteria, 78% of the ripened samples had *Pseudomonas* counts lower than 5 log cfu/g and 68% had *Enterobacteriaceae* counts lower than 4 log cfu/g. Thus, most of the samples have acceptable levels according to the guidelines for the microbiological quality of some ready-to-eat foods sampled at the point-of-sale (Gilbert et al. 2000).

CONCLUSION

This study highlighted the diversity observed in the manufacturing practices and the microbiota of naturally fermented traditional sausages from five Mediterranean countries (Spain, Italy, France, Greece and Portugal) and East-Central Europe (Slovakia). The diversity of practices led to a wide diversity of sausages. However the sausages can be discriminated along the process by LAB counts and a_w while *Enterobacteriaceae*, GCC+ and yeasts/moulds discriminated the ripened sausages according the geographical origin. Traditional dry fermented sausages did not present health risks in general, although the presence of some pathogens and spoilage microbiota in some sausages highlight the importance of maintaining sound hygienic procedures.

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