



MICROBIOTA OF COOKED HAM AND FRESH SAUSAGE PACKAGED IN MODIFIED ATMOSPHERE THROUGHOUT THE SHELF-LIFE

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The work focuses on the study of the microbiota of cooked ham and fresh sausage in MAP.

➤ A further understanding of the evolution of the endogenous microbiota of meat products during the shelf-life is a critical point to prevent premature spoilage and meet both safety and preservation standards.

An exhaustive overview about bacteria taxa other than LAB (Lactic Acid Bacteria) is still missing.

➢In this context, the research work investigates both the microbiological and chemical aspects of the products, combining culture-dependent and independent approaches.













## **EXPERIMENTAL DESIGN**

#### Fresh sausage processing - Workflow



Spoilage assessment

3 E samples out of 10 were still acceptable.

RESULTS









RESULTS

**Culture-dependent enumerations** 





On average, bacterial counts (log<sub>10</sub> cfu/g) increased as follows:

		-
	S	E
PCA	$4.7 \pm 0.6$	$6.6 \pm 0.8$
MRS	$3.7 \pm 0.5$	8.1 ± 1.0
BPA	$3.7 \pm 0.5$	5.5 ± 1.5
MAC	$3.0 \pm 0.4$	4.8 ± 1.6
CET	1.7 ± 0.3	$3.0 \pm 0.7$



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RESULTS



#### RAPD-PCR isolates at the end of the shelf-life

			М	RS										P	CA										_	٨								BPA						
	Lactobacillus curvatus/graminis (3)	Lactobacillus sakei (3)	Leuconostoc carnosum (1)	Leuconostoc kimchii (1)	Leuconostoc mesenteroides (2)	Bacillus sp. (1)	Bacillus siamensis $(I)$	Bacillus subtilis $(1)$	Bacillus velezensis (1)	Bacteroides vulgatus $(l)$	Brochothrix thermosphacta (0) 🏷	Carnobacterium divergens (4) 🏷	Carnobacterium gallimarum (2)	Everngella antericana $(l)$	Klupvera intermedia/cnyocrescens (2)	Lactobacillus curvatus/graminis (1)	Lactococcus garrieae (1)	Lelliottia annigena (1)	Leuconostoc carnosum (3) 🗙	Leuconostoc holzayfelii (1)	Leuconostoc kinchii (1)	Lenconostoc mesenteroides (3) 🗙	Macrococcus caseolyticus $(I)$	Pseudomonas moraviensis/koreensis $(l)$	Serratia liquefaciens (3)	Serratia proteamaculans/grimesti (3) 🛛 🗙	Serratia quinivorans (1)	Staphylococcus equorum (1)	Štaplijelococeus sciuri (1)	Staphylococcus vitulinus (2)	Staphylococcus xylosus (1)	Weissella hellenica $(l)$	Carnobacterium divergens (2)	Carnobacterium gallinarum (4)	Enterococcus faecalis (1)	Macrococcus caseolyticus (2)	Proteus vulgaris (1)	Staphylococcus equorum (2)	Staphylococcus sciuri (1)	Staphylococcus warneri (2) 🗙
Ole		96			4																	100												19	53		28			
02E	36				65						10				6	7			16			40		4							13	4			100					
03E	66	21			13	5	8	12				57					4			4	6	4											17	28		55				
04E	100										58	9	3	13	3			3				3			8								23		57			20		
05E	80	9		2	9						53														19	4	24						14			22		64		
06E	100										89														3	5			3					33	23			44		
07E	100										52		9							3			3			3		3		27			28	72						
08E			100								83	3							14															100						
09E	14		86																100																					100
10E	30	70							10	3	78		6														3									33		31	36	
All*	53	20	18	<1	9	<1	1	1	1	<1	42	7	2	1	1	1	<1	<1	13	1	1	15	<1	<1	3	1	3	<1	<1	3	1	<1	8	24	23	11	3	15	4	12
	* Val	lues v	vere c:	alculate	ed basin	e on th	ie tot:	าไ ทางห	nber (	ofiso	lates f	tom a	ll san	mles	in each	medi	um																							

#### RAPD-PCR isolates at the end of the shelf-life



Biotypes Brochotrix thermosphacta 6 Brochotrix thermosphacta 9 Carnobacterium divergens 12 Carnobacterium divergens 13 Carnobacterium gallinarum 18 Carnobacterium gallinarum 19 Enterococcus faecalis 20 Lactobacillus curvatus/graminis 24 Lactobacillus curvatus/graminis 25 Lactobacillus sakei 28 Leuconostoc carnosum 32 Leuconostoc mesenteroides 37 Macrococcus caseolyticus 41 Serratia liquefaciens 45 Serratia proteamaculans/grimesii 50 Serratia quinivorans 51 Staphylococcus equorum 52

RESULTS



#### **RESULTS** 16S rRNA gene profiling FRESH PORK SAUSAGE

A total of 792,402 quality-trimmed 16S rRNA gene sequences were obtained from 20 samples. 2041 OTUs net of singletons were identified.

**α-diversity** 

Start

Start







RESULTS

pH, organic acids, and biogenic amines



# RESULTS VOCs

#### FRESH PORK SAUSAGE





Conclusions

RESULTS

Data obtained using both cultural and not cultural methods are consistent.

➤ The 16S rRNA gene profiling revealed that initial microbiota was mainly composed of bacteria associated to the gut of slaughtered pigs (Firmicutes and Bacteroidetes). In E samples, although the presence of oxygen in MAP, facultative anaerobes such as Brochothrix and microaerophilic LAB dominated other taxa.

 $\succ$  The isolation and identification of cultivable strains highlighted the presence of a core microbiota recurrent in the different batches, suggesting a cross contamination of the meat within the production facility.





## **EXPERIMENTAL DESIGN**

#### Cooked ham processing - Workflow



**Overallevaluation** 

ID code	Analysis day	Slime/Colonies Discoloration	Sw ollen pack Sour smell/taste	Overallevaluation	ID code	Analyris day	Slime/Colonies Discoloration	ow ouen pack Sour smell/taste	<b>Overall evaluation</b>
01R	24	х	хх	в	12E	24			G
025	4				135	2			
02E	30		х	в					_
035	7				138	17	X	Х	в
03E	28		х	в	14S	2			
04R	32	хх	:	в :	14E	29	х	Х	В
058	3				15R	25	х		в
05E	32	х	х	в :	165	7			
06S	7				105	1			
06E	31		х	в	16E	28			G
07S	5				17S	3			
07E	30			G	17E	23	х		В
085	8				18S	2			
OSE	32	x x	x	в	18E	28		x	R
095	4								_
09E	44			G	19R	21		хх	в
10S	7								
10E	45	хх	x	в					
11R	49		хх	в					
125	11								



# RESULTS

#### Spoilage assessment





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Culture-dependent enumeration and pH



No relationship could be established between the LAB charge in S samples and the days elapsed from the date of packaging (R<sup>2</sup>=0.019).

**RESULTS** 



#### **RAPD-PCR** isolates

**19 species** (17 in S and 9 in E)

#### 7 genera

Carnobacterium

RESULTS

- Enterococcus
- Lactobacillus
- Leuconostoc
- Staphylococcus
- Streptococcus
- Weissella

#### Yeast species

- Candida sake
- Cryptococcus curvatus
- Yarrowia lipolytica
- Kazachstania servazzii

- Weissella viridescens
- Streptococcus oralis
- Streptococcus infantis
- Streptococcus australis
- Staphylococcus hominis
- Staphylococcus epidermidis
- Leuconostoc rapi
- Leuconostoc mesenteroides
- Leuconostoc carnosum
- Lactobacillus sakei
- Lactobacillus fuchuensis
- Lactobacillus curvatus
- Enterococcus raffinosus
- Enterococcus pseudoavium
- Enterococcus gilvus
- Enterococcus faecium
- Enterococcus faecalis
- Carnobacterium sp.
- Carnobacterium maltaromaticum



- 30% isolates (16 biotypes)
  *L.carnosum.*
- Other common species (<6% each one): *L.curvatus/graminis*, *W.viridescens*, *E.faecalis/gilvus*, *L.fuchuensis*).
- Streptococci and Staphylococci were limited to S samples.
- The most common yeast isolate was *C.sake.*







**RESULTS RAPD-PCR** isolates







05S 05E

06S

06E

THE REAL STREET RECORD

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#### RESULTS 16S rRNA gene profiling

#### COOKED HAM

A total of 415,225 quality-trimmed 16S rRNA gene sequences were obtained, clustered into 828 bacterial OTUs.

**α-diversity** 





Weissella

Xanthomonas

01R 04R 11R 15R 19R

0%

025 035 055 065 065 075 085 095 095 1105 1125 1135 1165 1175 1175 02E 03E 05E 06E 07E 08E 09E 112E 112E 112E 114E 116E 116E 117E 117E UNIVERSITÀ DEGLI STUDI DI MODENA E REGGIO EMILIA

PCo1 (48.6%)











This paper provides new knowledge on evolution of CH microbiota.

LAB dominated the microbiota, coherently with literature data, especially *L.sakei* and *L. carnosum*, which are here confirmed as relevant in lots from different countries.

➤The microbiota of EG and EB samples didn't show specific taxon that could be surely associated to spoilage, suggesting that it may be due to strain specificity or subdominant bacterial groups.

Consistently, it was not possible to associate specific patterns of VOCs to peculiar microbial groups or taxa.

> Data resulting from the culture dependent and independent approaches are consistent.





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Microbiota of sliced cooked ham packaged in modified atmosphere throughout the shelf life: Microbiota of sliced cooked ham in MAP

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# Thanks for your attention







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