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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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ABSTRACT

Fourteen lots of cooked ham in modified atmosphere packaging (CH) were analyzed within a few days from packaging (S) and at the end of the shelf-life (E), after storage at 7 °C to simulate thermal abuse. Five more lots, rejected from the market because spoiled (R), were included in the study. Quality of the products was generally compromised during the shelf life, with only 4 lots remaining unaltered. Analysis of 16S rRNA gene amplicons resulted in 801 OTUs. S samples presented a higher diversity than E and R ones. At the beginning of the shelf life, Proteobacteria and Firmicutes dominated the microbiota, with *Acinetobacter*, *Brochothrix*, *Carnobacterium*, *Lactobacillus*, *Prevotella*, *Pseudomonas*, *Psychrobacter*, *Weissella*, *Vibrio rumoiensis* occurring frequently and/or abundantly.

E and R samples were dominated by Firmicutes mostly ascribed to Lactobacillales. It is noteworthy the appearance of abundant *Leuconostoc*, negligible in S samples, in some E and R samples, while in other LAB were outnumbered by *V. rumoiensis* or *Brochothrix thermosphacta*. The microbiota of spoiled and R samples could not be clustered on the basis of specific defects (discoloration, presence of slime, sourness, and swollen packages) or supplemented additives. LAB population of S samples, averaging 2.9 $\log_{10}$(cfu/g), increased to 7.7 $\log_{10}$(cfu/g) in the E and R samples. Dominant cultivable LAB belonged to the species *Lactobacillus sakei* and *Leuconostoc carnosum*. The same biotypes ascribed to different species where often found in the corresponding S and R samples, and sometime in different batches provided from the same producer, suggesting a recurrent contamination from the plant of production. Consistently with growth of LAB, initial pH (6.26) dropped to 5.74 in E samples. Volatiles organic compound (VOCs) analysis revealed that ethanol was the major metabolite produced during the shelf life. The profile of volatile compounds got enriched with other molecules (e.g. 2-butanone, ethyl acetate, acetic acid, acetoin, butanoic acid, ethyl ester, butanoic acid, and 2,3-butanediol) mainly ascribed to microbial metabolism.

1. Introduction

Modified Atmosphere Packaging (MAP) is widely utilized to reduce the addition of exogenous preservatives and to extend the shelf life of food without altering the physical and chemical properties. However, sliced cooked ham in MAP (hereinafter referred to as CH) still represents a perishable product, very sensitive to bacterial spoilage (Borch et al., 1996; Samelis et al., 1998; Vasilopoulos et al., 2015). The

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Table 1
Spoilage assessment, pH, and viable counts on MRS and PDA of CH samples. The number of biotypes identified by RAPD-PCR, the species attribution, and the relative abundance are reported.

Sample[a]		Spoilage assessment[b]					pH	MRS																		PDA[c]						
ID code	Analysis day	Slime/Colonies	Discoloration	Swollen pack	Sour smell/taste	Overall evaluation		Log_{10}(cfu/g)	No. of biotypes	*Carnobacterium* sp.	*C. gallinarum*	*Enterococcus* sp.	*E. durans*	*E. faecalis*	*E. gilvus*	*E. malodoratus*	*L. curvatus/graminis*	*L. fuchuensis*	*L. sakei*	*Leuconostoc* sp.	*L. carnosum*	*L. mesenteroides*	*S. epidermidis*	*S. hominis*	*S. infantis*	*S. oralis*	*S. rubneri*	*W. viridescens*	Log_{10}(cfu/g)	No. of biotypes	*C. sake*	*C. curvatus*	*K. servazzii*	*Y. lipolytica*
01R	24	X		X	X	B	5.69	8.5	4												100%								1.5	1	100%			
02S	4						6.27	1.2	5		12%					12%					39%		25%	12%									
02E	30				X	B	5.58	8.6	2								21%				79%								2	1	100%			
03S	7						6.10	1.8	3								62%				38%													
03E	28				X	B	5.73	8.1	2								94%				6%								4.3	1		100%		
04R	32	X	X			B	5.52	7.4	3												100%								<1.0	-				
05S	3						6.22	3.2	3										100%															
05E	32	X			X	B	5.59	8.8	4										100%										<1.0	-				
06S	7						6.22	6	3										100%															
06E	31				X	B	5.14	8.6	2										100%										<1.0	-				
07S	5						6.19	3.8	2										96%		4%													
07E	30					G	6.00	7.8	2										8%									92%	<1.0	-				
08S	8						6.20	4	4										100%															
08E	32	X	X		X	B	5.50	7.2	5										98%									2%	<1.0	-				
09S	4						6.22	1.2	5	7%									67%						7%	7%	14%							
09E	44					G	5.60	7.7	2										100%										<1.0	-				
10S	7						6.18	1.9	3						4%				30%		66%													
10E	45	X	X		X	B	5.68	7.6	1						100%														<1.0	-				
11R	49			X	X	B	5.59	8.4	3										94%	6%									<1.0	-				
12S	11						7.09	2.9	5										13%		68%							19%						
12E	24					G	6.16	8.4	2										4%		96%								<1.0	-				
13S	2						6.93	2.2	3									2%	4%		94%													
13E	17	X			X	B	6.06	7.5	3									4%	13%		83%								<1.0	-				
14S	2						6.16	4	2				49%						51%															
14E	29	X			X	B	5.39	7.8	1										100%										<1.0	-				
15R	25	X				B	5.48	6.4	1										100%										3.9	1	100%			
16S	7						6.05	2.2	5												90%	5%	5%											
16E	28					G	6.06	6.2	2										90%			10%							<1.0	-				
17S	3						6.17	4.2	1									100%																
17E	23	X				B	5.76	6.3	1												100%								7.2	3	72%		25%	3%
18S	2						6.60	1.6	1			100%																						
18E	28				X	B	6.10	6.6	2			16%		84%															<1.0	-				
19R	21			X	X	B	5.98	7.6	2										100%										<1.0	-				

[a] Samples were given an ID composed by the batch number followed by a letter: S, analyzed at the start of shelf life; E, analyzed closed to the sell-by date; R, rejected from the market. Analysis days are the days elapsed from the packaging date.
[b] Spoilage evaluated on E and R samples; B, Bad; G, Good; X, observed defect.
[c] Counts on PDA were done only for sample E and R; – no biotype.

main undesired defects caused by microbial growth include pH decrease, gas and slime production, blowing, discoloration, purge, and off-flavors formation. In some cases, the defects can result in the premature spoilage and in the reduction of shelf-life (Korkeala and Björkroth, 1997).

Manufacturing of cooked ham ends with cooking at a core temperature up to 70 °C, thus killing most of vegetative microbes. However, CH undergoes a massive bacterial proliferation toward the end of the shelf life despite the combination of hygienic precautions, preservative procedures such as chilling, micro-aerophilic conditions, and presence of NaCl and nitrites. The bacterial community in CH can reach 10^7–10^9 cfu/ g in few weeks after packaging, with a composition depending on many factors, including packaging, gas atmosphere, product composition, hygienic conditions throughout the processing line, and the temperature during both the distribution and the storage in consumer fridge (Audenaert et al., 2010; Kreyenschmidt et al., 2010; Samelis et al., 1998; Vasilopoulos et al., 2008). Previous studies reported a preferential growth of psychrotrophic lactic acid bacteria (LAB), with *Leuconostoc carnosum*, *Leuconostoc gelidum*, *Lactobacillus sakei*, *Lactobacillus curvatus*, *Carnobacterium divergens*, *Carnobacterium maltaromaticum* as the most recurrent species, which dominated the bacterial community at the end of the shelf life (Audenaert et al., 2010; Geeraerts et al., 2017; Vasilopoulos et al., 2008). Nonetheless, information on the microbiota composition in CH is still scarce with respect to the bacterial groups other than LAB.

In order to obtain a wide description of the microbiota composition and diversity, CH samples coming from 10 producers in 6 European countries were analyzed. They presented different characteristics in terms of MAP composition, time of storage, and presence of additives. Microbiota was studied throughout the shelf life of CH by 16S rRNA gene profiling and by a culture-dependent method to specifically isolate and trace the LAB biotypes, with the perspective to select candidates for CH biopreservation. Dominant LAB were isolated, genotyped by RAPD-PCR fingerprinting, and were given a taxonomic designation by partial sequencing of 16S rRNA gene. CH samples were analyzed at the packaging time and at the end of shelf life, during which they were stored at 7 °C. The set of samples included also a group of CH that were rejected as spoiled. For all the samples, the occurrence of defects was evaluated and the prevalent volatile compounds (VOCs) were determined by solid phase micro extraction (SPME) coupled with GC–MS.

2. Materials and methods

2.1. Sample collection and experimental design

Eleven commercial CH were received from 10 producers spread over 6 European countries. The additives and the gas mixture, composed by N_2 and CO_2, are reported in Table S1. As a whole, 19 batches of production