International Journal of Food Microbiology 289 (2019) 189–199

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Mapping the dominant microbial species diversity at expiration date of raw meat and processed meats from equine origin, an underexplored meat ecosystem, in the Belgian retail

Wim Geeraerts, Luc De Vuyst, Frédéric Leroy*

Research Group of Industrial Microbiology and Food Biotechnology (IMDO), Faculty of Sciences and Bioengineering Sciences, Vrije Universiteit Brussel, Pleinlaan 2, B-1050 Brussels, Belgium

ARTICLE INFO

Keywords:
Horse
Zebra
Smoked
Fermented
Lactic acid bacteria
Staphylococci, enterobacteria

ABSTRACT

Although equine meats and their derived smoked or fermented products are popular in some regions of the world, they only form a minor fraction of the global meat consumption. The latter may explain why their associated bacterial communities have not received much attention. In the present study, 69 different samples of equine meats and meat products were investigated. The samples consisted of raw meat from horses (17 samples) and zebra (7), as well as non-fermented but smoked (24) and fermented (21) horse meat products. After purchase, all samples were stored at 4 °C and analysed at expiration date. Besides an estimation of the total microbial counts, specific attention was paid to the identification of lactic acid bacteria (LAB) and catalase-positive cocci, in particular the group of coagulase-negative staphylococci (CNS), involved, due to their technological relevance in view of the elaboration of meat products. Samples that were loosely wrapped in butcher paper instead of vacuum- or modified-atmosphere packages were also screened for pseudomonads and enterobacterial species. In total, 1567 bacterial isolates were collected, subjected to $(GTG)_5$-PCR fingerprinting of genomic DNA, and identified by multiple gene sequencing (based on the 16S rRNA, *pheS*, *rpoA*, *rpoB*, and/or *tuf* genes). Overall, the bacterial species diversity consisted mostly of LAB but was contingent on the type of product. Raw meat was dominated by *Carnobacterium divergens*, *Lactobacillus sakei*, *Lactococcus piscium*, and *Leuconostoc gelidum*, with zebra meat being particularly rich in lactococci. Smoked and fermented horse meat products contained mostly *Lb. sakei* and, to a lesser degree, *Lactobacillus curvatus*. In addition, several catalase-positive cocci (mostly *Staphylococcus equorum*), *Anoxybacillus* sp., *Brevibacterium* sp., *Brochothrix thermosphacta*, and the enterobacterial species *Hafnia alvei* were found.

1. Introduction

Horse meat is part of the gastronomic and cultural heritage of many regions of Europe and Asia, albeit that it is often subjected to cultural (*e.g.*, in the Anglosphere) and religious (*e.g.*, in Jewish communities) taboos (Bell, 2015; Kelekna, 2009; Kesse et al., 2005; Zuckerman, 2013). Ritual slaughtering of horses was performed by some ancient cultures, including the ancient Chinese and several Celtic and Germanic tribes (Nam et al., 2010). Moreover, horse meat products are sometimes looked favourably upon, based on perceived health benefits compared to other meat types, due to the low cholesterol and fat contents and the high levels of proteins, omega-3 fatty acids, and iron (Lorenzo et al., 2014). The fact that methane production associated with horse meat production is lower than that for cattle may also lead to environmental benefits (Belaunzaran et al., 2015). From an ecological perspective, it has been argued that the consumption of horse meat may help to deal with the damaging overabundance of wild horses in the American West and in Australia (Chan, 2014; Enders, 2015). Nevertheless, horse meat only makes up a minor fraction of the world's meat production. In 2013, a total of 785,000 t was produced globally, whereas total meat production exceeded 310,000,000 t, corresponding with an average global horse meat supply of 0.10 kg *per capita* per year (Belaunzaran et al., 2015; FAO, 2018). In the European Union, several countries are known to produce and consume horse meat. In Belgium, horse meat consumption has historical roots and is still relatively widespread nowadays. The Belgian horse meat supply is estimated at 0.58 kg *per capita* per year. Products sold on the Belgian market include raw horse meat and non-fermented but smoked and/or fermented derivatives, which have a traditional image and importance. Besides horse meat, another type of equine meat can sometimes be found in the Belgian retail,

* Corresponding author.
E-mail address: frederic.leroy@vub.be (F. Leroy).

https://doi.org/10.1016/j.ijfoodmicro.2018.09.019
Received 18 June 2018; Received in revised form 6 September 2018; Accepted 21 September 2018
Available online 22 September 2018
0168-1605/

namely zebra meat, albeit only as a restricted niche product (Anonymous, 2014).

The most popular meats and meat products worldwide, in particular beef, pork, and poultry, have often been subjected to analyses of their dominant microbial communities, in view of hygienic control (*e.g.*, Doulgeraki et al., 2012; Geeraerts et al., 2017, 2018; Vasilopoulos et al., 2008). In contrast, only little research has been done on less commonly consumed meats, such as goat, game, and equine meats (*e.g.*, Borilova et al., 2016; Russo et al., 2017). Fresh goat meat, for instance, contains lactic acid bacteria (LAB) and *Pseudomonas* spp. (Carrizosa et al., 2017). For equine products, the microbiota of raw foal meat packed under vacuum or modified atmosphere consists of different LAB species, besides some pseudomonads and enterobacterial species (Lorenzo and Gómez, 2012). In spontaneously acidified Belgian horse meat sausage ('Boulogne'), the dominant microorganisms consist of LAB species (mostly *Lactobacillus sakei*, besides *Leuconostoc* spp.) and coagulase-negative staphylococci (CNS) species (Janssens et al., 2012). Chinese fermented horse meat contains, in addition to the applied starter culture of *Lb. sakei* and *Staphylococcus xylosus*, several LAB species (*Enterococcus faecium*, *Lactobacillus plantarum,* and *Weissella hellenica*), *Staphylococcus carnosus*, *Enterobacter cloacae*, and *Pseudomonas* sp. (Lu et al., 2015). Although the use of starter cultures usually supresses bacterial species diversity during meat fermentation, the specificities of the processing environment can profoundly affect the pervasiveness of starter culture species in the end products. For instance, a too high or low pH may lead to the vanishing of *S. carnosus* (Stavropoulou et al., 2018) or *S. xylosus* (Ravyts et al., 2010), respectively. To the authors' knowledge, microbiological studies on Belgian horse meat products are scarce and little is known about the effects of starter culture application (Janssens et al., 2012).

The present study endeavours to obtain a better insight into the microbial species diversity associated with different types of equine meats and their derived products. Rather than to evaluate microbial community dynamics during production or as a result of different storage conditions in a specific product, the aim is to more broadly map the diversity in a broad selection of end products that are representative for Belgian retail. To this end, several samples of raw, non-fermented but smoked, and fermented products were collected and subjected to microbiological analysis at expiration date. Focus was on the total microbiota and, more specifically, on the LAB and CNS communities because of their potential technological importance. As such, both LAB and CNS may be of interest for the potential future development of starter cultures for appropriate meat fermentation (Ravyts et al., 2012), whereas LAB can also serve as bioprotective cultures for applications in non-fermented meat and meat products (Vasilopoulos et al., 2015).

2. Materials and methods

2.1. Sample acquisition

A total of 69 samples was purchased at three local supermarkets and six butcher shops in the agglomeration of Brussels between May 2015 and March 2017 (Tables 1–3; Table S1), being representative for conventional practice in Belgian retail. The samples consisted of raw meat derived from horse (17 non-frozen samples) and zebra (3 non-frozen and 4 frozen samples), as well as several processed horse meat products (24 non-fermented but smoked and 21 fermented samples, respectively). Of the raw horse meats, the samples purchased at the butcher shops (8) were loosely wrapped in paper, whereas the remaining horse and zebra meats were obtained from supermarkets as vacuum-packed products. More than half of the fermented (12 of 21 samples) and non-fermented, smoked (16 of 24 samples) horse meat products were stored under modified-atmosphere packaging (MAP) or vacuum and obtained from supermarkets, whereas the remaining ones were wrapped in butcher paper and purchased at the butcher shops. All MAP-, vacuum-, and paper-packaged samples were stored at 4 °C until the expiration date was reached. At expiration date (all supermarket samples; expiration date as stated on the label) or two days after acquisition (all butcher samples), all samples were checked for microbial growth and the pH was measured with an InoLab pH 7110 meter (WTW, Weilheim, Germany). All samples were verified for sensory defects based on visual inspection and odour evaluation.

2.2. General microbiological sample analysis

Dilution series of all equine product samples were made as described before (Geeraerts et al., 2017). Prior to a medium-strength mechanical treatment of 90 s with a Stomacher 400 (Seward, Worthington, UK), a mass of 10–20 g of equine meat was put in a stomacher bag and diluted ten times using a peptone physiological solution (0.85%, m/v, NaCl and 0.1%, m/v; peptone in ultrapure water). Dilution series were made and spread plating was carried out using brain heart infusion (BHI) agar medium (Oxoid, Basingstoke, Hampshire, UK) to estimate total microbial counts, de Man-Rogosa-Sharpe (MRS) agar medium (Oxoid) for presumable LAB, and mannitol salt-phenol red-agar (MSA; VWR International, Darmstadt, Germany) for presumable CNS. Dilution series of paper-packaged samples were plated on RAPID Enterobacteriaceae agar medium (Biorad, Marnes-La-Laquette, France) for presumable enterobacterial species and Pseudomonas agar base (PAB; Oxoid) with added Pseudomonas CFC supplement (Oxoid) for presumable pseudomonads. The BHI and MRS agar media were incubated at 22 °C for 120 h, MSA media were incubated at 30 °C for 96 h, and RAPID agar and PAB media were incubated at 25 °C for 5 days.

2.3. Isolation and identification of microbial species

Isolates were picked up randomly (10–30% of the total number of colonies) from the highest sample dilutions, transferred into the appropriate liquid media, and stored in cryovials, the media being supplemented with 25% (v/v) of glycerol, at −80 °C. DNA extraction and $(GTG)_5$-PCR fingerprinting of genomic DNA was done as described previously (Geeraerts et al., 2017). Briefly, BHI and MRS agar isolates were grown in BHI broth at 22 °C and MSA isolates at 30 °C for 24–48 h. Cell pellets were generated by a 5-min centrifugation step at 13,793 $\times g$ (Heraeus Biofuge 13, Thermo Fisher Scientific, Waltham, MA, USA), followed by a wash step with TES buffer (0.05 M Tris-base; 0.001 M EDTA; 0.2 M sucrose; pH 8.0), and a dual lysis step, first with a mixture of lysozyme (Merck, Darmstadt, Germany) and mutanolysin (Sigma-Aldrich, St. Louis, MO, USA) at 37 °C for 60 min, and then with proteinase K (Macherey-Nagel, Düren, Germany) at 56 °C for 60 min. The extraction and purification of genomic DNA was performed using the NucleoSpin®96 Tissue kit (Macherey-Nagel), according to the manufacturer's instructions. The DNA concentrations were determined with a Nanodrop 2000 (Thermo Fisher Scientific), followed by $(GTG)_5$-PCR fingerprinting as described previously (Vasilopoulos et al., 2008). Bionumerics software (v. 5.10; Applied Maths, Sint-Martens-Latem, Belgium) was used for the classification and identification of the isolates, based on numerical cluster analysis of the $(GTG)_5$-PCR fingerprints obtained (Geeraerts et al., 2017). Representative isolates of the different clusters were identified by 16S rRNA gene sequencing (Vasilopoulos et al., 2008). The identity of the LAB stains was confirmed by sequencing of the *pheS* gene (De Bruyne et al., 2007; Snauwaert et al., 2013). Further identification to species level of *Staphylococcus* and *Kocuria* isolates was done by amplification of the *rpoB* and *tuf* genes using the rpoB-F (5'-AACCAATTCCGTATIGGTTT-3′) and rpoB-R (5'-CCGTCCCAAGTCATGAAAC-3′) primers (Drancourt and Raoult, 2002) and the tuf-F (5'-GCCAGTTGAGGACGTATTCT-3′) and tuf-R (5'-CCATTTCAGTACCTTCTGGTAA-3′) primers (Heikens et al., 2005), respectively. Secondary identification of the enterobacterial isolates was done by sequencing of the *rpoA* gene, using the rpoA_entro-L (5'-ATGCAGGGTTCTGTGACAGAG-3′) and rpoA_entro-R (5'-GGTGGCCARTTTTCYAGGCGC-3′) primers (Kuhnert et al., 2009). The gene

Table 1

Overview of the raw equine meat products with their specifications of origin (B, butcher shop; I, production facility delivering to supermarkets), product type (R), batch, packaging method, salt concentration, bacterial counts on different agar media (BHI agar, MRS agar, MSA, RAPID agar, and PAB), sample pH, and the number of isolates belonging to each species, including *Anoxybacillus* sp., *Brevibacterium* sp., *Brochothrix thermosphacta*, *Carnobacterium divergens*, *Hafnia alvei*, *Kocuria* sp., *Lactobacillus curvatus*, *Lactobacillus fuchuensis*, *Lactobacillus sakei*, *Lactococcus piscium*, *Lactococcus plantarum*, *Leuconostoc carnosum*, *Leuconostoc gelidum* subsp. *gelidum*, *Leuconostoc mesenteroides*, *Staphylococcus equorum*, *Staphylococcus saprophyticus*, *Staphylococcus similans*, and *Staphylococcus xylosus*. Accession numbers can be found in the supplementary Table 4S.

Origin	Product type	Batch	Packaging method	Salt concentration (%)	Bacterial counts [log (cfu g^{-1})]					pH	Number of isolates per species			
					BHI agar	MRS agar	MSA	RAPID agar	PAB		*Anoxybacillus* sp.	*Brevibacterium* sp.	*B. thermosphacta*	*C. divergens*
I1	R1	A	Vacuum	0.13	7.1	7.3	2.5	n.a.[a]	n.a.	5.43	0	0	0	3
		B	Vacuum	0.13	6.5	6.2	3.0	n.a.	n.a.	5.58	0	0	0	15
		C	Vacuum	0.13	7.2	7.2	< 2.0	n.a.	n.a.	5.42	0	0	0	1
		D	Vacuum	0.13	7.6	7.3	< 2.0	n.a.	n.a.	5.29	0	0	0	0
I2	R2	A	Vacuum	0.10	7.0	6.9	2.4	n.a.	n.a.	5.52	0	0	0	13
		B	Vacuum	0.10	7.2	6.5	2.5	n.a.	n.a.	5.46	0	8	0	6
		C	Vacuum	0.10	4.0	3.6	3.8	n.a.	n.a.	5.64	0	0	0	1
I3	R3	A	Vacuum	n.a.	7.3	7.2	< 2.0	n.a.	n.a.	5.39	0	0	0	2
		B	Vacuum	n.a.	7.2	7.3	< 2.0	n.a.	n.a.	5.12	0	0	0	4
I4	R4	A	Vacuum	0.00	7.2	7.3	< 2.0	n.a.	n.a.	5.86	1	0	0	5
		B	Vacuum	0.00	7.8	6.4	2.9	n.a.	n.a.	5.85	0	0	0	6
		C	Vacuum	0.00	7.0	7.0	< 2.0	n.a.	n.a.	5.66	0	0	3	0
	R5[b]	A	Vacuum	0.00	< 2.0	< 2.0	< 2.0	n.a.	n.a.	5.95	0	0	0	0
		B	Vacuum	0.00	< 2.0	< 2.0	< 2.0	n.a.	n.a.	6.62	0	0	0	0
		C	Vacuum	0.00	< 2.0	< 2.0	< 2.0	n.a.	n.a.	6.05	0	0	0	0
		D	Vacuum	0.00	< 2.0	< 2.0	< 2.0	n.a.	n.a.	5.75	0	0	0	0
B1	R6	A	Paper	n.a.	6.8	6.6	3.6	2.3	2.0	5.73	0	2	0	0
		B	Paper	n.a.	6.3	6.3	2.9	3.5	3.1	5.64	0	0	0	0
B2	R7	A	Paper	n.a.	6.5	6.6	2.1	< 2.0	< 2.0	5.57	0	0	0	1
		B	Paper	n.a.	4.9	4.7	3.6	< 2.0	< 2.0	5.62	0	0	0	0
		C	Paper	n.a.	5.9	6.0	< 2.0	2.6	2.4	5.76	0	0	1	6
B3	R8	A	Paper	n.a.	7.7	7.6	2.2	2.4	2.5	5.42	0	0	0	4
		B	Paper	n.a.	7.5	7.4	2.6	< 2.0	< 2.0	5.38	0	0	0	2
		C	Paper	n.a.	7.3	7.4	3.3	6.4	5.2	5.81	0	0	0	0

Origin	Number of isolates per species													
	H. alvei	*Kocuria* sp.	*Lb. curvatus*	*Lb. fuchuensis*	*Lb. sakei*	*Lc. piscium*	*Lc. plantarum*	*Leuc. carnosum*	*Leuc. gelidum* subsp. *gelidum*	*Leuc. mesenteroides*	*S. equorum*	*S. saprophyticus*	*S. similans*	*S. xylosus*
I1	0	0	0	0	5	4	2	0	0	0	4	0	0	0
	0	0	1	0	9	0	2	1	0	0	6	0	0	0
	0	0	0	0	3	0	0	0	10	0	0	0	0	0
	0	0	0	0	10	5	0	0	0	0	0	1	0	0
I2	0	0	0	0	7	0	0	0	0	0	0	0	0	0
	0	0	0	1	4	0	0	0	0	0	0	0	0	1
	0	12	0	3	7	0	0	0	1	0	0	1	1	5
I3	0	0	0	1	0	1	0	0	0	0	0	0	0	0
	0	0	0	1	2	1	0	0	0	0	0	0	0	0
I4	0	0	0	0	0	5	2	0	1	0	0	0	0	0
	0	0	0	1	1	4	7	1	10	0	0	0	0	0
	0	0	0	3	0	2	2	1	4	1	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0

(continued on next page)

Table 1 (*continued*)

Origin	Number of isolates per species													
	H. alvei	*Kocuria* sp.	*Lb. curvatus*	*Lb. fuchuensis*	*Lb. sakei*	*Lc. piscium*	*Lc. plantarum*	*Leuc. carnosum*	*Leuc. gelidum* subsp. *gelidum*	*Leuc. mesenteroides*	*S. equorum*	*S. saprophyticus*	*S. similans*	*S. xylosus*
B1	0	0	0	0	1	0	0	0	2	0	2	5	0	0
	6	0	0	0	0	0	0	0	0	0	7	0	0	0
B2	0	0	0	4	0	0	0	0	3	0	5	9	0	1
	0	0	0	1	0	1	0	0	7	0	9	3	0	1
	0	0	0	2	0	1	0	0	0	0	0	0	0	0
B3	0	0	0	0	17	1	0	0	0	0	1	1	0	0
	0	0	0	0	18	1	0	0	0	0	0	2	0	1
	30	0	7	0	23	4	0	0	0	0	2	0	0	0

[a] n.a., not available.
[b] All samples of product type R5 were purchased frozen at −20 °C.

sequencing results were processed using the BioEdit Sequence Alignment Editor (v. 7.2.5.0; Ibis Biosciences, Carlsbad, CA, USA) and identities were assigned with the basic local alignment search tool (BLAST) and the national center for biotechnology information (NCBI) database (http://www.ncbi.nlm.nih.gov/BLAST).

2.4. Statistics

Unpaired two-sample Student's *t*-tests were carried out to compare data where appropriate. Analyses were performed using Microsoft Excel.

3. Results

3.1. General microbiological analysis

The pH values of all equine meat (product) samples investigated ranged between 5.12 and 6.62, 5.26 and 5.81, and 4.51 and 5.00 for the raw, non-fermented but smoked, and fermented products, respectively (Tables 1–3). Raw zebra meat had a higher average pH value (5.79 ± 0.11) than raw horse meat (5.43 ± 0.16) ($p = 0.003$). The average bacterial counts amounted to 6.5 ± 1.3 log cfu g^{-1} on BHI agar, 6.5 ± 1.3 log cfu g^{-1} on MRS agar, 3.2 ± 1.2 log cfu g^{-1} on MSA, 2.9 ± 1.5 log cfu g^{-1} on RAPID agar, and 2.7 ± 1.3 log cfu g^{-1} on PAB. On both BHI and MRS agar, the bacterial counts varied substantially, mostly being situated between 4.0 and 9.0 log cfu g^{-1}, with only the four frozen raw zebra meat samples displaying counts below 2.0 log cfu g^{-1}. On MSA, the bacterial counts from the non-fermented, smoked meat samples (mostly around 4.0 to 5.0 log cfu g^{-1}) were generally somewhat higher than those of the raw meat samples (mostly 2.0 to 3.0 log cfu g^{-1}) ($p < 0.001$). With the exception of two and four samples, respectively, the bacterial counts of the samples packed in butcher paper on RAPID agar and PAB were below 2.0 log cfu g^{-1}. The differences in bacterial counts among the various media and per product type are suggestive of differences in prevalence of bacterial groups, which needs to be further addressed by analysing the species diversity.

To this end, a total of 1567 bacterial isolates was picked up from the different agar media (Tables 1–3). Most of these isolates were identified as LAB (69.6%) and catalase-positive cocci (25.8%). The LAB consisted of the genera *Lactobacillus* (54.4% of the total isolates), *Carnobacterium* (6.3%), *Leuconostoc* (5.6%), and *Lactococcus* (3.3%). Within the LAB communities, *Lactobacillus* represented the most prevalent genus (78.2% of all LAB isolates), of which *Lactobacillus sakei* was by far the most retrieved species (68.4% of all LAB isolates), largely exceeding the presence of *Lactobacillus curvatus* (7.5%) and *Lactobacillus fuchuensis* (2.2%). Other LAB species identified were *Carnobacterium divergens* (8.5% of all LAB isolates), *Carnobacterium maltaromaticum* (0.6%) *Lactococcus plantarum* (1.5%), *Lactococcus piscium* (3.2%), *Leuconostoc carnosum* (1.3%), *Leuconostoc gelidum* subsp. *gelidum* (4.6%), and *Leuconostoc mensenteroides* (2.2%). Within the *Staphylococcus* genus, *Staphylococcus equorum* (36.1% of all staphylococcal isolates) and *Staphylococcus carnosum* (32.2%) were the most prevalent species. Other species identified were *Staphylococcus saprophyticus* (8.4% of all staphylococcal isolates), *Staphylococcus simulans* (19.2%), and *Staphylococcus xylosus* (4.1%). A minor fraction of the enterobacterial species *Hafnia alvei* (3.3% of the total isolates) was retrieved from RAPID agar and PAB only, originating from two raw meat (B1R6B and B3R8C) and two non-fermented, smoked meat samples (B5S10A and B5S10C). Also, minor fractions (below 1.0%) of the total isolates were composed of *Anoxybacillus* sp., *Brochothrix thermosphacta*, *Brevibacterium* sp., and *Kocuria* sp.

3.2. Microbiological analysis according to product type

3.2.1. Raw equine meat

In general, the bacterial counts on BHI and MRS agar media were

Table 2
Overview of the non-fermented, smoked equine meat products with their specifications of origin (B, butcher shop; I, production facility delivering to supermarkets), product type (S), batch, packaging method, salt concentration, bacterial cell counts on different agar media (BHI agar, MRS agar, MSA, RAPID agar, and PAB agar), sample pH, and the number of isolates belonging to each species, including *Anoxybacillus* sp., *Brevibacterium* sp., *Brochothrix thermosphacta*, *Carnobacterium divergens*, *Carnobacterium maltaromaticum*, *Hafnia alvei*, *Kocuria* sp., *Lactobacillus curvatus*, *Lactobacillus fuchuensis*, *Lactobacillus sakei*, *Lactococcus piscium*, *Lactococcus plantarum*, *Leuconostoc carnosum*, *Leuconostoc gelidum* subsp. *gelidum*, *Leuconostoc mesenteroides*, *Staphylococcus carnosum*, *Staphylococcus equorum*, *Staphylococcus saprophyticus*, *Staphylococcus similans* and *Staphylococcus xylosus*. Accession numbers can be found in the supplementary Table 4S.

Origin	Product type	Batch	Packaging method	Salt concentration (%)	Bacterial counts [log (cfu g^{-1})]					pH	Number of isolates per species				
					BHI agar	MRS agar	MSA	RAPID agar	PAB		*Anoxybacillus* sp.	*Brevibacterium* sp.	*B. thermosphacta*	*C. divergens*	*C. maltaromaticum*
I2	S5	A	MAP	2.9	7.0	7.1	< 2.0	n.a.[a]	n.a.	5.64	0	0	0	1	6
		B	MAP	2.9	7.2	7.2	3.0	n.a.	n.a.	5.75	0	0	0	2	0
	S6	A	MAP	3.1	6.9	6.9	2.4	n.a.	n.a.	5.62	0	1	0	5	0
		B	MAP	3.1	9.2	8.8	2.7	n.a.	n.a.	5.79	0	0	0	7	0
		C	MAP	3.1	7.2	7.3	2.0	n.a.	n.a.	5.46	0	0	0	0	0
		D	MAP	3.1	7.1	7.0	2.8	n.a.	n.a.	5.91	0	0	0	0	0
I5	S1	A	MAP	3.9	7.1	6.8	< 2.0	n.a.	n.a.	5.63	0	0	0	0	0
	S2	A	MAP	3.1	7.1	7.1	< 2.0	n.a.	n.a.	5.45	0	0	0	0	0
		B	MAP	3.1	7.1	7.2	4.4	n.a.	n.a.	5.23	0	0	0	0	0
I6	S3	A	MAP	3.1	7.2	7.1	< 2.0	n.a.	n.a.	5.65	0	0	0	0	0
		B	MAP	3.1	6.9	7.0	< 2.0	n.a.	n.a.	5.42	0	0	0	0	0
		C	MAP	3.1	7.8	7.6	< 2.0	n.a.	n.a.	5.74	1	0	0	0	0
I7	S4	A	MAP	3.0	7.0	6.8	5.3	n.a.	n.a.	5.68	0	0	0	0	0
I8	S7	A	MAP	3.2	6.9	6.9	5.7	n.a.	n.a.	5.59	0	0	0	0	0
		B	MAP	3.2	6.9	6.8	5.0	n.a.	n.a.	5.52	0	0	0	0	0
		C	MAP	3.2	7.0	6.9	6.4	n.a.	n.a.	5.60	0	0	0	0	0
B1	S8	A	Paper	n.a.	7.1	7.1	4.6	< 2.0	< 2.0	5.69	0	0	0	0	0
		B	Paper	n.a.	6.9	6.9	5.3	< 2.0	< 2.0	5.58	1	0	0	0	0
		C	Paper	n.a.	6.9	6.8	3.9	< 2.0	< 2.0	5.43	0	0	0	1	0
		D	Paper	n.a.	6.8	6.8	4.5	< 2.0	< 2.0	5.26	1	0	0	0	0
B4	S9	A	Paper	n.a.	6.8	6.9	4.3	< 2.0	< 2.0	5.78	0	0	0	1	0
B5	S10	A	Paper	n.a.	7.2	7.2	5.7	< 2.0	5.1	5.41	1	0	0	6	0
		B	Paper	n.a.	6.3	6.5	5.0	< 2.0	2.6	5.78	0	0	1	0	0
		C	Paper	n.a.	6.9	6.8	6.2	2.1	5.3	5.81	0	0	0	0	0

Origin	Number of isolates per species														
	H. alvei	*Kocuria* sp.	*Lb. curvatus*	*Lb. fuchuensis*	*Lb. sakei*	*Lc. plantarum*	*Lc. piscium*	*Leuc. carnosum*	*Leuc. gelidum* subsp. *gelidum*	*Leuc. mesenteroides*	*S. carnosus*	*S. equorum*	*S. saprophyticus*	*S. similans*	*S. xylosus*
I2	0	1	0	0	10	0	0	0	0	2	0	3	0	0	0
	0	0	0	2	19	0	0	0	0	2	0	1	0	0	0
	0	0	0	2	5	0	0	0	7	1	0	3	0	0	0
	0	0	0	0	18	0	2	0	0	0	0	1	0	0	0
	0	0	0	1	15	0	0	0	0	1	0	0	4	6	0
	0	0	1	0	23	0	0	0	0	0	0	1	0	1	0
I5	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	15	0	0	1	0	3	3	0	1	0	0
	0	0	2	0	10	0	0	0	0	5	0	0	0	15	0
I6	0	0	0	0	16	0	0	0	0	0	0	0	0	0	0
	0	0	1	0	12	0	0	0	0	1	0	0	0	0	0
	0	0	0	0	5	0	0	4	0	0	0	0	0	0	0
I7	0	0	1	0	11	0	0	3	0	0	0	13	0	0	0
I8	0	0	1	0	12	0	0	0	0	0	0	0	1	12	0
	0	0	4	0	12	0	0	0	0	0	0	0	0	7	0
	0	0	5	0	13	0	0	0	0	1	0	0	0	16	0

(continued on next page)

Table 2 (*continued*)

Origin	Number of isolates per species														
	H. alvei	*Kocuria* sp.	*Lb. curvatus*	*Lb. fuchuensis*	*Lb. sakei*	*Lc. plantarum*	*Lc. piscium*	*Leuc. carnosum*	*Leuc. gelidum* subsp. *gelidum*	*Leuc. mesenteroides*	*S. carnosus*	*S. equorum*	*S. saprophyticus*	*S. similans*	*S. xylosus*
B1	0	0	18	0	4	0	0	0	0	0	0	11	0	0	0
	0	0	13	0	6	0	0	0	0	0	0	13	1	0	0
	0	0	1	2	11	0	2	0	0	0	9	0	0	0	0
	0	0	0	0	26	0	0	0	0	4	0	4	0	0	0
B4	0	0	2	0	13	1	0	1	0	2	0	0	0	6	5
B5	3	0	18	0	7	0	0	1	1	0	0	9	0	0	0
	0	0	0	0	21	0	0	1	0	0	0	9	2	0	0
	13	0	0	0	9	0	0	0	0	0	0	19	0	0	0

[a] n.a., not available.

higher than those on MSA ($p < 0.001$), RAPID ($p < 0.001$), and PAB agar media ($p < 0.001$), thus representing the dominant microbiota. Comparing the horse meat samples from butchers and supermarkets revealed some differences in microbial species diversity (Fig. 1; Table 2S). The samples of horse meat that were purchased at the supermarkets, which were all vacuum-packed, resulted mostly in isolates belonging to the genus *Lactobacillus* (*i.e.*, 38.0% and 73.9% of all BHI and MRS agar isolates retrieved from raw horse meat samples acquired at supermarkets, respectively), whereas *Carnobacterium* (22.0% and 4.3%, respectively), *Lactococcus* (20.0% and 6.5%, respectively), and *Leuconostoc* (12.0% and 13.0%, respectively) were present too. For the samples purchased at the butcher shops, the isolates from BHI and MRS agar media mostly yielded LAB species, with *Lb. sakei* (45.8% and 57.4% of all BHI and MRS agar isolates retrieved from raw horse meat samples acquired at butcher shops) being the most prevalent. Other retrieved LAB on BHI and MRS agar media species from butcher's horse meat were *C. divergens* (12.5% and 5.6%, respectively), *Lb. curvatus* (4.2% and 9.3%, respectively), *Lc. piscium* (10.4% and 5.6%, respectively), and *Leuc. gelidum* subsp. *gelidum* (6.3% and 11.1%, respectively).

When analysing the total set of MSA isolates that originated from raw horse meat obtained at supermarket level, most of the isolates belonged to the genera *Carnobacterium* (46.4%) and *Staphylococcus* (24.6%). It has to be pointed out that carnobacteria were recovered from MSA as small colonies, indicating suboptimal growth on this medium. The samples purchased at the butcher shops mostly led to the isolation of *Staphylococcus* spp., consisting of 73.2% of all the MSA isolates retrieved from raw horse meat samples acquired at butcher level. Within the group of CNS, *S. equorum* was the main species, representing 42.9% of the total set of MSA isolates.

A comparison of vacuum-packed raw horse meat with non-frozen vacuum-packed zebra meat showed that both were dominated by LAB species. The genus *Lactobacillus* prevailed on the horse meat products (38.0% and 73.9% of all BHI and MRS agar isolates, respectively). Alternatively, lactobacilli were hardly present on the non-frozen vacuum-packed zebra meat (21.0% as L. *fuchuensis*, and 0.0% of all BHI and MRS agar isolates, respectively), which was dominated by *Leuc. gelidum* subsp. *gelidum* (0.0% and 62.5%, respectively), *Lc. pisicum* (31.6% and 20.8%, respectively), and *Lc. plantarum* (36.8% and 4.2%, respectively). With respect to the presence of enterobacterial species, only *H. alvei* was found. More specifically, the species was recovered from two butcher raw horse meat samples (B1R6B and B3R8C), reaching relatively high levels on RAPID agar for the latter one (6.4 log cfu g^{-1}). The B3R8C was also indicative of spoilage based on odour deviations.

3.2.2. Processed equine meats

The processed equine meats analysed consisted of different non-fermented but smoked and fermented horse meat products (Fig. 2; Table 3S). Isolates retrieved from BHI and MRS agar consisted primarily of *Lactobacillus* spp., both for samples from butcher shops (90.7% and 92.4% of all BHI and MRS agar isolates, respectively) and supermarkets (90.6% and 88.7% of all BHI and MRS agar isolates, respectively). Although *Lb. sakei* was the primary LAB species from both butcher and supermarket samples, it was more common in the supermarket samples (85.9% and 83.0% of all BHI and MRS agar, respectively) than in the ones from butcher shops (74.0% and 78.6% of all BHI and MRS agar, respectively), where also *Lb. curvatus* was found (16.0% and 13.1%, respectively).

The isolates from MSA mainly belonged to the genus *Staphylococcus* for both the butcher and supermarket samples (71.0% and 78.0% of the MSA isolates, respectively). While the supermarket samples were dominated by *S. carnosum* (40.0% of the MSA isolates retrieved from the processed horse meat samples) and *S. similans* (26.0%), the samples purchased at the butcher shops were dominated by *S. carnosum* (37.0% of the MSA isolates) and *S. equorum* (34.0%).

Table 3
Overview of the fermented equine meat products with their specifications of origin (B, butcher shop; I, production facility delivering to supermarkets), product type (R), batch, packaging method, salt concentration, bacterial cell counts on different agar media (BHI agar, MRS agar, MSA, RAPID agar, and PAB agar), sample pH, and the number of isolates belonging to each species, including *Carnobacterium divergens*, *Lactobacillus curvatus*, *Lactobacillus sakei*, *Lactococcus piscium*, *Leuconostoc gelidum* subsp. *gelidum*, *Leuconostoc mesenteroides*, *Staphylococcus carnosum*, *Staphylococcus equorum*, *Staphylococcus saprophyticus*, *Staphylococcus similans* and *Staphylococcus xylosus*. Accession numbers can be found in the supplementary Table 4S.

Origin	Product type	Batch	Packaging method	Salt concentration (%)	Bacterial counts [log (cfu g^{-1})]					pH
					BHI agar	MRS agar	MSA	RAPID agar	PAB	
I5	F1	A	MAP	n.a.[a]	6.4	6.4	< 2.0	n.a.	n.a.	4.59
		B	MAP	n.a.	6.8	6.8	3.5	n.a.	n.a.	4.70
		C	MAP	n.a.	6.7	6.3	3.6	n.a.	n.a.	4.72
		D	MAP	n.a.	6.8	6.8	3.7	n.a.	n.a.	4.86
I6	F2	A	MAP	3.6	5.3	5.4	3.7	n.a.	n.a.	4.75
I8	F4	A	MAP	3.6	6.1	5.1	2.7	n.a.	n.a.	4.84
		B	MAP	3.6	6.1	6.0	4.3	n.a.	n.a.	4.62
		C	MAP	3.6	6.7	6.5	2.8	n.a.	n.a.	4.51
		D	MAP	3.6	6.2	6.3	3.9	n.a.	n.a.	4.59
		E	MAP	3.6	6.1	6.1	3.1	n.a.	n.a.	4.65
I9	F3	A	Vacuum	3.6	7.5	7.5	4.0	n.a.	n.a.	4.89
		B	Vacuum	3.6	7.3	6.3	3.1	n.a.	n.a.	4.89
B1	F5	A	Paper	n.a.	6.0	6.0	4.1	3.6	2.3	5.00
		B	Paper	n.a.	6.7	6.7	3.2	< 2.0	< 2.0	4.76
		C	Paper	n.a.	5.9	5.8	3.7	< 2.0	< 2.0	4.82
B5	F7	A	Paper	n.a.	7.2	7.1	4.3	< 2.0	< 2.0	4.87
		B	Paper	n.a.	6.4	6.4	4.0	< 2.0	< 2.0	4.78
		C	Paper	n.a.	5.7	5.7	4.0	< 2.0	2.1	4.92
B6	F6	A	Paper	n.a.	7.0	6.7	3.7	< 2.0	< 2.0	4.90
		B	Paper	n.a.	7.1	7.0	3.9	< 2.0	< 2.0	4.98
		C	Paper	n.a.	6.2	6.0	3.2	< 2.0	< 2.0	4.78

Origin	Number of isolates per species										
	C. divergens	*Lb. curvatus*	*Lb. sakei*	*Lc. piscium*	*Leuc. gelidum* subsp. *gelidum*	*Leuc. mesenteroides*	*S. carnosus*	*S. equorum*	*S. saprophyticus*	*S. similans*	*S. xylosus*
I5	0	1	17	0	0	0	1	0	0	0	0
	0	1	19	0	0	0	0	0	0	0	0
	0	0	16	0	0	0	7	0	0	0	0
	0	0	12	0	0	0	6	0	0	0	0
I6	1	1	7	1	0	0	0	0	0	0	0
I8	0	1	12	0	0	0	11	0	1	2	0
	0	1	16	0	0	0	11	0	0	0	0
	0	0	14	0	0	0	0	0	0	2	0
	0	0	21	0	0	0	0	0	0	0	0
	0	0	17	0	0	0	12	0	0	0	0
I9	0	0	16	0	0	0	12	0	0	0	0
	0	0	31	0	0	0	5	0	0	1	0
B1	0	0	12	0	0	0	13	0	0	0	0
	0	1	12	0	0	0	2	0	1	0	0
	0	0	11	0	0	0	0	0	0	0	0
B5	0	0	20	0	0	0	8	4	0	1	0
	0	0	21	0	2	0	7	4	0	3	2
	0	0	13	0	2	1	0	7	0	2	0

(*continued on next page*)

Table 3 (*continued*)

Origin	Number of isolates per species										
	C. divergens	*Lb. curvatus*	*Lb. sakei*	*Lc. piscium*	*Leuc. gelidum* subsp. *gelidum*	*Leuc. mesenteroides*	*S. carnosus*	*S. equorum*	*S. saprophyticus*	*S. similans*	*S. xylosus*
B6	0	0	13	0	0	0	12	0	0	0	0
	0	0	26	0	0	0	6	1	0	0	0
	0	1	14	0	0	0	1	2	0	0	0

[a] n.a., not available.

When comparing the isolates of the two types of processed horse meats, both types mainly consisted of mixtures of LAB and CNS. On both product types, *Lb. sakei* was the most prevalent LAB species, constituting 46.7% and 67.9% of all non-fermented but smoked and fermented meat isolates from all agar media, respectively. On non-fermented, smoked products, *Lb. curvatus* was also present (11.5% of all non-fermented, smoked horse meat isolates). Within the CNS communities, *S. carnosus* was the most prevalent species on fermented horse meat products, representing 22.8% of all the isolates from all agar media. On the smoked horse meat products, *S. equorum* was found to be the most prevalent species, corresponding with 15.2% of all the isolates from all agar media. The enterobacterial species *H. alvei* was found as a very minor fraction of the isolates from PAB media obtained from two non-fermented, smoked equine meat samples (B5S10A and B5S10C).

4. Discussion

The microbiomes of equine meats and their derived products have been largely underexplored, despite the fact that they are prone to microbial spoilage (Lorenzo and Gómez, 2012; Lorenzo et al., 2017). The occurrence of *Kocuria* sp., *Lb. sakei*, *Leuc. carnosum*, *Leuc. mesenteroides*. *S. carnosus*, *S. saprophyticus*, and *S. xylosus* on these products, as in the present study, has been explicitly associated with horse meat in the past (Janssens et al., 2012; Lu et al., 2015). However, the other species found, such as *Anoxybacillus* sp., *Brevibacterium* sp., *Brochothrix thermosphacta*, *C. divergens*, *C. maltaromaticum*, *H. alvei*, *Lb. curvatus*, *Lc. lactis*, *Lc. pisicum*, *Leuc. gelidum* subsp. *gelidum*, *S. equorum*, and *S. similans*, have mostly been described in the context of other raw meats and/or meat products (Audenaert et al., 2010; Björkroth et al., 2005; Chajęcka-Wierzchowska et al., 2015; Ercolini et al., 2006; Geeraerts et al., 2017; Rahkila et al., 2012; Ratsimba et al., 2017; Vasilopoulos et al., 2008).

The LAB consortia pertaining to the raw equine meats consisted of carnobacteria, lactobacilli, lactococci, and leuconostocs, which are habitually known to colonize chilled vacuum-packed pork and beef as well (Dušková et al., 2015; Jiang et al., 2010). When compared to the samples purchased at the supermarkets, raw meat from butchers was somewhat more dominated by lactobacilli. Whether this is intrinsic to the butcher context is unclear, but a certain level of cross-contamination from cutting equipment may be presupposed. Among the CNS, *S. equorum* was the most retrieved isolate from raw horse meat, which is not surprising as it was first found on the skin of healthy horses and can thus be transferred through the processing chain onto the final product (Schleifer et al., 1984). In comparison to horse meat, on which *Lactobacillus* and *Carnobacterium* acted as the dominant LAB genera, *Lactococcus* and *Leuconostoc* were the most prevalent LAB representatives on non-frozen zebra meat samples. It is not known at this stage whether this finding has to be related to the specific characteristics of zebra meat or to the fact that all zebra meat samples originated from the same production facility, possibly involving a specific house microbiota. From frozen zebra meat, no microbial isolates could be retrieved, which may be ascribed to the fact that these samples were already stored for more than two years at $-20\,^{\circ}C$ in the retail. As a result, the meat-associated microbiota may have been either dead or occurred in a viable-but-non-culturable state (Abd El-Aziz et al., 2018).

Non-fermented, smoked meat products were largely dominated by lactobacilli, with *Lb sakei* being the most prevalent species. The prevalence of lactobacilli in smoked meat products also been shown for other products, including cooked turkey meat products, among which the smoked products were dominated by *Lb. sakei* and the non-smoked variants by *Leuconostoc* spp. and *Weissella viridescens* (Samelis et al., 2000). Dominance of *Lb. sakei* in smoked products may be partially due to the presence of polycyclic aromatic hydrocarbons (PAHs), which are transferred into the meat matrix during the smoking process (Djinovic et al., 2008). Certain *Lb. sakei* strains are known to be capable of degrading PAHs, hypothetically explaining their dominance on the

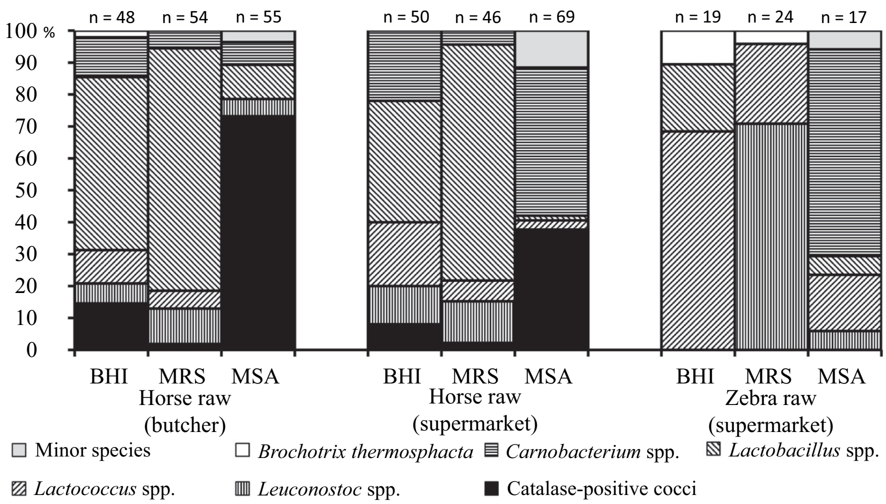

Fig. 1. Percentage distribution of the bacterial isolates from raw equine meats retrieved from BHI agar, MRS agar, and MSA, as a function of the origin of acquisition (butcher *versus* supermarket) and the animal species (horse *versus* zebra). *Anoxybacillus* sp. and *Brevibacterium* sp. are grouped under 'Minor species'; *Kocuria* sp. and *Staphylococcus* spp. are grouped under 'Catalase-positive cocci'. Accession numbers can be found in the supplementary Table 4S.

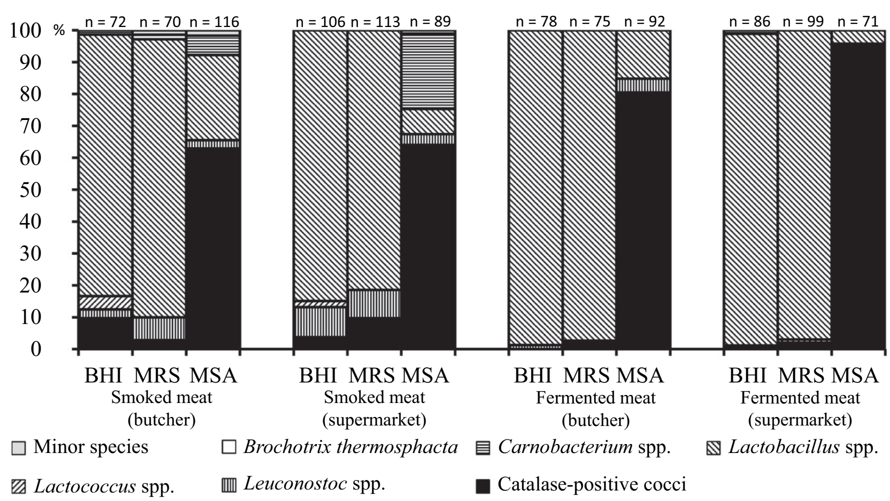

Fig. 2. Percentage distribution of the bacterial isolates from processed equine meat products retrieved from BHI agar, MRS agar, and MSA, as a function of their production process (non-fermented but smoked *versus* fermented) and location of acquisition (butcher *versus* supermarket), *Anoxybacillus* sp. and *Brevibacterium* sp. are grouped under 'Minor species'; *Kocuria* sp. and *Staphylococcus* spp. are grouped under 'Catalase-positive cocci'. Accession numbers can be found in the supplementary Table 4S.

smoked products (Bartkiene et al., 2017). Moreover, the latter species is very well adapted not only to meat as a substrate (Rimaux et al., 2011a, 2011b) but also to high salt concentrations (Leroy and De Vuyst, 1999). In smoked horse meat products, the relatively high salt content of 2.9–3.9% (m/m) may thus have acted as a major microbial inhibitor, thereby selecting for certain LAB species (Taormina, 2010). Potentially, the origin of *Lb. sakei* could also be linked to its common use as a starter culture for meat fermentation (Ravyts et al., 2012), so that some cross-contamination within production facilities producing both non-fermented, smoked and fermented products can be expected. Such effects are not uncommon when different types of meat products are handled within the same environment, especially when slicing is involved (Dušková et al., 2015; Gormley et al., 2010; Srey et al., 2013; Vasilopoulos et al., 2015). Contamination through contact surfaces generally occurs in food processing companies (Bokulich and Mills, 2013).

The microbial species diversity of the fermented horse meat products consisted mostly of a mixture of *Lb. sakei* and *S. carnosum*, which were likely to originate from the use of starter cultures (Janssens et al., 2012; Ravyts et al., 2012; Sánchez Mainar et al., 2017). During meat fermentation, LAB are used as acidifiers, whereas CNS play a technological role related to the formation and stabilization of colour and flavour (Sánchez Mainar et al., 2017). Their counts, however, were rather on the low side on the fermented equine products, mostly in the order of 3.0 to 4.0 log cfu g^{-1}, when compared to other fermented meat products wherein they often achieve about 5.0 to 7.0 log cfu g^{-1}, provided that the pH drop is not too drastic (Fonseca et al., 2013; Janssens et al., 2012; Samelis et al., 1994). This may be tentatively connected with the rather strong acidification profile of the fermented horse meat products investigated, as CNS are usually rather sensitive to low pH values (Leroy et al., 2017; Sánchez Mainar et al., 2014; Van Netten et al., 1998).

In conclusion, the bacterial ecology of raw, non-fermented but smoked, and fermented equine meats and meat products was generally dominated by LAB species. Nonetheless, differences in species prevalence were noticed as a function of the type of product technology (smoking *versus* fermenting), the storage and packaging method (vacuum-packed *versus* packaging in butcher paper), and the animal species from which the meat was obtained (horse *versus* zebra). Further research should investigate to which degree the compositional particularities of equine meats and their derived products play a role in creating a selective ecological context, resulting in specific microbial adaptations and community variations. Also, the effects of critical manufacturing steps during the production of equine meat products, such as smoking or starter culture use, merit further exploration.

Acknowledgements

The authors acknowledge their financial support of the Research Council of the Vrije Universiteit Brussel (SRP7, and IOF342 projects, and in particular the HOA21 project 'Artisan quality of fermented foods: myth, reality, perceptions, and constructions' and the Interdisciplinary

Research Program IRP2 'Food quality, safety, and trust since 1950: societal controversy and biotechnological challenges') and the Hercules Foundation (project UABR 09/004).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijfoodmicro.2018.09.019.

References

Abd El-Aziz, N.K., Tartor, Y.H., Gharib, A.A., Ammar, A.M., 2018. Propidium monoazide quantitative real-time polymerase chain reaction for enumeration of some viable but nonculturable foodborne bacteria in meat and meat products. Foodborne Pathog. Dis. 15, 226–234.

Anonymous, 2014. Zebraburger nieuwe Belgische primeur van Cook & Roll. Het Laatste Nieuws. https://www.hln.be/regio/oostende/zebraburger-nieuwe-belgische-primeur-van-cook-roll~a3dfff5f.

Audenaert, K., D'Haene, K., Messens, K., Ruyssen, T., Vandamme, P., Huys, G., 2010. Diversity of lactic acid bacteria from modified atmosphere packaged sliced cooked meat products at sell-by date assessed by PCR-denaturing gradient gel electrophoresis. Food Microbiol. 27, 12–18.

Bartkiene, E., Bartkevics, V., Mozuriene, E., Krungleviciute, V., Novoslavskij, A., Santini, A., Rozentale, I., Juodeikiene, G., Cizeikiene, D., 2017. The impact of lactic acid bacteria with antimicrobial properties on biodegradation of polycyclic aromatic hydrocarbons and biogenic amines in cold smoked pork sausages. Food Control 71, 285–292.

Belaunzaran, X., Bessa, R.J.B., Lavín, P., Mantecón, A.R., Kramer, J.K.G., Aldai, N., 2015. Horse-meat for human consumption - current research and future opportunities. Meat Sci. 108, 74–81.

Bell, R., 2015. Kazakhstan: where horses are revered and eaten. In: National Geographic, . http://theplate.nationalgeographic.com/2015/12/17/kazakhstan-where-horses-are-revered-and-eaten.

Björkroth, J., Ristiniemi, M., Vandamme, P., Korkeala, H., 2005. *Enterococcus* species dominating in fresh modified-atmosphere-packaged, marinated broiler legs are overgrown by *Carnobacterium* and *Lactobacillus* species during storage at 6 °C. Int. J. Food Microbiol. 97, 267–276.

Bokulich, N.A., Mills, D.A., 2013. Facility-specific "House" microbiome drives microbial landscapes of artisan cheesemaking plant. Appl. Environ. Microbiol. 79, 5214–5223.

Borilova, G., Hulankova, R., Svobodova, I., Hutarova, Z., Vecerek, V., Steinhauserova, I., 2016. The effect of storage conditions on the hygiene and sensory status of wild boar meat. Meat Sci. 118, 71–77.

Carrizosa, E., Benito, M.J., Ruiz-Moyano, S., Hermández, A., Villabobos, C., Martin, A., De Guía Córdoba, M., 2017. Bacterial communities of fresh goat meat packaged in modified atmosphere. Food Microbiol. 66, 57–63.

Chajęcka-Wierzchowska, W., Zadernowska, A., Nalepa, B., Sierpińska, M., Laniewska-Trokenheim, L., 2015. Coagulase-negative staphylococci (CoNS) isolated from ready-to-eat food of animal origin - phenotypic and genotypic antibiotic resistance. Food Microbiol. 46, 222–226.

Chan, G., 2014. A time to cull? The battle over Australia's brumbies. In: The Guardian, . https://www.theguardian.com/world/2014/aug/20/-sp-a-time-to-cull-the-battle-over-australias-brumbies.

De Bruyne, K., Schillinger, U., Caroline, L., Boehringer, B., Cleenwerck, I., Vancanneyt, M., De Vuyst, L., Franz, C.M.A.P., Vandamme, P., 2007. *Leuconostoc holzapfelii* sp. nov., isolated from Ethiopian coffee fermentation and assessment of sequence analysis of housekeeping genes for delineation of *Leuconostoc* species. Int. J. Syst. Evol. Microbiol. 57, 2952–2959.

Djinovic, J., Popovic, A., Jira, W., 2008. Polycyclic aromatic hydrocarbons (PAHs) in different types of smoked meat products from Serbia. Meat Sci. 80, 449–456.

Doulgeraki, A.I., Ercolini, D., Villani, F., Nychas, G.-J.E., 2012. Spoilage microbiota associated to the storage of raw meat in different conditions. Int. J. Food Microbiol. 157, 130–141.

Drancourt, M., Raoult, D., 2002. *RpoB* gene sequence-based identification of *Staphylococcus* species. J. Clin. Microbiol. 40, 1333–1338.

Dušková, M., Kameník, J., Šedo, O., Zdráhal, Z., Saláková, A., Karpíšková, R., Lačanin, I., 2015. Survival and growth of lactic acid bacteria in hot smoked dry sausages (non-fermented salami) with and without sensory deviations. Food Control 50, 804–808.

Enders, C., 2015. Why you really should (but really can't) eat horsemeat. In: The Guardian, . https://www.theguardian.com/environment/2015/jan/09/eating-wild-horsemeat-america.

Ercolini, D., Russo, F., Torrieri, E., Masi, P., Villani, F., 2006. Changes in the spoilage-related microbiota of beef during refrigerated storage under different packaging conditions. Appl. Environ. Microbiol. 72, 4663–4671.

FAO, 2018. Livestock Primary. http://www.fao.org/faostat/en/#data/QL.

Fonseca, S., Cachaldora, A., Gómez, M., Franco, I., Carballo, J., 2013. Monitoring the bacterial population dynamics during the ripening of Galician chorizo, a traditional dry fermented Spanish sausage. Food Microbiol. 33, 77–84.

Geeraerts, W., Pothakos, V., De Vuyst, L., Leroy, F., 2017. Diversity of the dominant bacterial species on sliced cooked pork products at expiration date in the Belgian retail. Food Microbiol. 65, 236–243.

Geeraerts, W., Pothakos, V., De Vuyst, L., Leroy, F., 2018. Variability within the dominant microbiota of sliced cooked poultry products at expiration date in the Belgian retail. Food Microbiol. 73, 209–215.

Gormley, F.J., Little, C.L., Grant, K.A., de Pinna, E., McLauchlin, J., 2010. The microbiological safety of ready-to-eat specialty meats from markets and specialty food shops: a UK wide study with a focus on *Salmonella* and *Listeria monocytogenes*. Food Microbiol. 27, 243–249.

Heikens, E., Fleer, A., Paauw, A., Florijn, A., Fluit, A.C., 2005. Comparison of genotypic and phenotypic methods for species-level identification of clinical isolates of coagulase-negative staphylococci. J. Clin. Microbiol. 43, 2286–2290.

Janssens, M., Myter, N., De Vuyst, L., Leroy, F., 2012. Species diversity and metabolic impact of the microbiota are low in spontaneously acidified Belgian sausages with an added starter culture of *Staphylococcus carnosus*. Food Microbiol. 29, 167–177.

Jiang, Y., Gao, F., Xu, X.L., Su, Y., Ye, K.P., Zhou, G.H., 2010. Changes in the bacterial communities of vacuum-packaged pork during chilled storage analyzed by PCR–DGGE. Meat Sci. 86, 889–895.

Kelekna, P., 2009. The politico-economic impact of the horse on old world cultures: an overview. In: Sino-platonic Papers. 190. pp. 1–31.

Kesse, E., Boutron-Ruault, M.C., Clavel-Chapelon, F., 2005. Regional dietary habits of French women born between 1925 and 1950. Eur. J. Nutr. 44, 285–292.

Kuhnert, P., Korczak, B.M., Stephan, R., Joosten, H., Iversen, C., 2009. Phylogeny and prediction of genetic similarity of *Cronobacter* and related taxa by multilocus sequence analysis (MLSA). Int. J. Food Microbiol. 136, 152–158.

Leroy, F., De Vuyst, L., 1999. The presence of salt and a curing agent reduces bacteriocin production by *Lactobacillus sakei* CTC 494, a potential starter culture for sausage fermentation. Appl. Environ. Microbiol. 65, 5350–5356.

Leroy, S., Vermassen, A., Ras, G., Talan, R., 2017. Insight into the genome of *Stapylococcus xylosus*, a ubiquitous species well adapted to meat products. Microorganisms 5, 1–18.

Lorenzo, J.M., Gómez, M., 2012. Shelf life of fresh foal meat under MAP, overwrap and vacuum packaging conditions. Meat Sci. 92, 610–618.

Lorenzo, J.M., Sarriés, M.V., Tateo, A., Polidori, P., Franco, D., Lanza, M., 2014. Carcass characteristics, meat quality and nutritional value of horsemeat: a review. Meat Sci. 96, 1478–1488.

Lorenzo, J.M., Munekata, P.E.S., Bastianello Campagnol, P.C., Zhu, Z., Alpas, H., Barba, F.J., Tomasevic, I., 2017. Technological aspects of horse meat products - a review. Food Res. Int. 102, 176–183.

Lu, S., Ji, H., Wang, Q., Li, K., Xu, C., Jiang, C., 2015. The effects of starter cultures and plant extracts on the biogenic amine accumulation in traditional Chinese smoked horsemeat sausages. Food Control 50, 869–875.

Nam, K.C., Jo, C., Lee, M., 2010. Meat products and consumption culture in the east. Meat Sci. 86, 95–102.

Rahkila, R., Nieminen, T., Johansson, P., Säde, E., Björkroth, J., 2012. Characterization and evaluation of the spoilage potential of *Lactococcus piscium* isolates from modified atmosphere packaged meat. Int. J. Food Microbiol. 156, 50–59.

Ratsimba, A., Leroy, S., Chacornac, J.P., Rakoto, D., Arnoud, E., Jeannoda, V., Talon, R., 2017. Staphylococcal ecosystem of kitoza, a traditional malagasy meat product. Int. J. Food Microbiol. 246, 20–24.

Ravyts, F., Steen, L., Goemaere, O., Paelinck, H., De Vuyst, L., Leroy, F., 2010. The application of staphylococci with flavour-generating potential is affected by acidification in fermented dry sausages. Food Microbiol. 27, 945–954.

Ravyts, F., De Vuyst, L., Leroy, F., 2012. Bacterial diversity and functionalities in food fermentations. Eng. Life Sci. 12, 356–367.

Rimaux, T., Vrancken, G., Pothakos, V., Maes, D., De Vuyst, L., Leroy, F., 2011a. The kinetics of the arginine deiminase pathway in the meat starter culture *Lactobacillus sakei* CTC 494 are pH-dependent. Food Microbiol. 28, 597–604.

Rimaux, T., Vrancken, G., Vuylsteke, B., De Vuyst, L., Leroy, F., 2011b. The pentose moiety of adenosine and inosine is an important energy source for the fermented-meat starter culture *Lactobacillus sakei* CTC 494. Appl. Environ. Microbiol. 77, 6539–6550.

Russo, C., Balloni, S., Altomonte, I., Martini, M., Nuvoloni, N., Cecchi, F., Pedonese, F., Salari, F., Sant'ana Da Silva, A.M., Torracca, B., Profumo, A., 2017. Fatty acid and microbiological profile of the meat (*Longissimus dorsi* muscle) of wild boar (*Sus scropha scropha*) hunted in Tuscany. Ital. J. Anim. Sci. 16, 1–8.

Samelis, J., Stravropoulos, S., Kakouri, A., Metaxopoulos, J., 1994. Quantification and characterization of microbial populations associated with naturally fermented Greek dry salami. Food Microbiol. 11, 447–460.

Samelis, J., Kakouri, A., Rementzis, J., 2000. The spoilage microflora of cured, cooked turkey breasts prepared commercially with or without smoking. Int. J. Food Microbiol. 56, 133–143.

Sánchez Mainar, M., Weckx, S., Leroy, F., 2014. Coagulase-negative staphylococci favor conversion of arginine into ornithine despite a widespread genetic potential for nitric oxide synthase activity. Appl. Environ. Microbiol. 80, 7741–7751.

Sánchez Mainar, M., Stravropoulou, D.A., Leroy, F., 2017. Exploring the metabolic heterogeneity of coagulase-negative staphylococci to improve the quality and safety of fermented meats: a review. Int. J. Food Microbiol. 247, 24–37.

Schleifer, K.H., Kilpper-Bälz, R., Devriese, L.A., 1984. *Staphylococcus arlettae* sp. nov., *S. equorum* sp. nov. and *S. kloosii* sp. nov.: three new coagulase-negative, novobiocin-resistant species from animals. Syst. Appl. Microbiol. 5, 501–509.

Snauwaert, I., Hoste, B., De Bruyne, K., Peeters, K., De Vuyst, L., Willems, A., Vandamme, P., 2013. *Carnobacterium iners* sp. nov., a psychrophilic, lactic acid-producing bacterium from the littoral zone of an Antarctic pond. Int. J. Syst. Evol. Microbiol. 63, 1370–1375.

Srey, S., Jahid, I.K., Ha, S.D., 2013. Biofilm formation in food industries: a food safety concern. Food Control 31, 572–585.

Stavropoulou, D.A., De Maere, H., Berardo, A., Janssens, B., Filippou, P., De Vuyst, L., De Smet, S., Leroy, F., 2018. Pervasiveness of *Staphylococcus carnosus* over *Staphylococcus xylosus* is affected by the level of acidification within a conventional meat starter culture set-up. Int. J. Food Microbiol. 274, 60–66.

Taormina, P.J., 2010. Implications of salt and sodium reduction on microbial food safety. Crit. Rev. Food Sci. Nutr. 50, 209–227.
Van Netten, P., Valentijn, A., Mossel, D.A.A., Huis in't Veld, J.H.J., 1998. The survival and growth of acid-adapted mesophilic pathogens that contaminate meat after lactic acid decontamination. J. Appl. Microbiol. 84, 559–567.
Vasilopoulos, C., Ravyts, F., De Maere, H., De Mey, E., Paelinck, H., De Vuyst, L., Leroy, F., 2008. Evaluation of the spoilage lactic acid bacteria in modified-atmosphere-packaged artisan-type cooked ham using culture-dependent and culture-independent approaches. J. Appl. Microbiol. 104, 1341–1353.
Vasilopoulos, C., De Vuyst, L., Leroy, F., 2015. Shelf-life reduction as an emerging problem in cooked hams underlines the need for improved preservation strategies. Crit. Rev. Food Sci. Nutr. 55, 1425–1443.
Zuckerman, C., 2013. Europe's horse meat scandal casts light on food taboo. Natl. Geogr. https://news.nationalgeographic.com/news/2013/13/130212-horse-meat-beef-scandal-food-france-england-europe-science-taboo-horsemeat.