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General patterns of background microbiota and selected bacterial pathogens during production of fermented sausages in Serbia

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The presence of *Salmonella* spp. or *Escherichia coli* O157 and background microbiota, pH and a_w were determined in raw fermented sausages produced from pork or beef and without lactic acid bacteria starters. The investigation was conducted at five meat processing plants, and the sampling was done at five steps of the production process at each plant. In meat trimmings, total viable count (TVC) ranged around 6 log CFU/g and around 5–6 log CFU/g in the pork and the beef sausages, respectively. *Enterobacteriaceae* count (EBC) ranged in the vicinity of 3–4 log CFU/g, whilst *E. coli* count (ECC) ranges were comparably lower (by 1–2 logs). During chopping of both the pork and the beef trimmings, the levels of TVC, EBC and ECC increased by 1–1.5 logs. After the additives and the spices were added, background microbiota tended to slightly decrease, generally more noticeably in pork sausages and with ECC. During the fermentation-drying stage, in both pork and beef sausages, initial TVC levels (6–7 log CFU/g) increased by the mid-process (by approximately 1.5–2 logs) and remained at those levels in finished products. During the same period, lactic acid bacteria (LAB) increased from initial levels of 5.5–6 log CFU/g to around 7–8 log CFU/g in pork and around 8–9 log CFU/g in beef sausages, and became the predominant microbial group. *Salmonella* spp. was found in the first three stages of the production process (trimmings, trimmings chopping, mixing with additives/spices), in two of three meat processing plants, but not at later stages of the production process. *E. coli* O157 was found only in one sample of chopped trimmings in one meat processing plant. The background microbiota patterns and levels were, generally, similar to those commonly reported for raw fermented sausages in other published studies. The initial presence of foodborne pathogens in raw fermented sausage production may be considered as a potential meat safety risk, because in the case of high initial pathogen counts, their total elimination cannot be assumed.

1. Introduction

Raw, dry, fermented sausages (further: fermented sausages) are products with a long shelf life. Their shelf life relies on biochemical and physicochemical changes in fermentation and drying processes which create a hostile environment for spoilage and pathogenic microorganisms. However, it is well known that fermented sausages sometimes contain bacterial foodborne hazards, i.e. pathogens originating primarily from incoming raw materials including meat and fatty tissue (Nørrung & Buncic, 2008). The combination of antimicrobial factors acting in fermented sausages (e.g. low pH, antagonistic lactic acid microbiota, low water activity) can prevent growth and, at best, reduce the counts of bacterial pathogens. The traditional production process of fermented sausages leads to an appreciable reduction in pathogen counts (Adams & Mitchell, 2002; Nightingale, Thippareddi, Phebus, Marsden, & Nutsch, 2006), but the total elimination of pathogens cannot be ensured in all cases. In the last two decades, epidemiological investigations have shown that fermented sausages, as the main vehicles for foodborne pathogens, have been involved in several foodborne disease outbreaks (Ethelberg et al., 2009; Gieraltowski et al., 2013; Kuhn, Torpdahl, Frank, Sigsgaard, & Ethelberg, 2011; Luzzi et al., 2007; Sekse et al., 2009).

Verotoxigenic *Escherichia coli* (VTEC), especially O157:H7 serotype, can cause bloody diarrhoea and life-threatening hemolytic-uraemic syndrome at low infectious doses in humans. Cattle are the

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principle reservoir for VTEC, and so consequently, this pathogen can contaminate carcass meat during cattle slaughter as well as the fermented sausages produced from such beef (Nastasijevic, Mitrovic, & Buncic, 2009). Hence, VTEC represent an important health hazard associated with beef fermented sausages (Holck et al., 2011). This risk is potentiated by the facts that VTEC, particularly *E. coli* O157:H7, appear to be relatively acid tolerant and, as the infectious dose can be low, the pathogen's growth in contaminated foods is not necessary to cause associated foodborne illness (Sartz et al., 2008). On the other hand, *Salmonella* spp. is one of the major causes of foodborne diseases in humans, and after eggs and poultry meat, pork is regarded as the most important source of foodborne salmonellosis. As *Salmonella* is associated with fresh pork, it may be present also in raw fermented sausages containing pork; consumption of which is relatively common in a number of countries (EFSA, 2006). The *Salmonella* risk posed by pork fermented sausages is potentiated by the pathogen's ability to grow in foods with a water activity down to 0.94 (or sometimes even lower) or with pH > 4 (Nørrung, Andersen, & Buncic, 2009). Further, *Salmonella* and *E. coli* O157 can originate not only from raw meat/fatty tissue, but also from other ingredients such as spices (Gieraltowski et al. 2013; Holck et al. 2011). Consequently, raw fermented sausages may represent a significant microbial food safety risk for consumers. Fermented sausages are one of the most popular types of meat products in Serbia, and are also potentially very important export items, but published information on *Salmonella* and *E. coli* O157 in these Serbian products is very limited or is completely lacking, respectively.

Therefore, the main aim of the present study was to find if the most relevant bacterial foodborne pathogens occur in pork (*Salmonella*) and beef (*E. coli* O157) raw fermented sausages throughout their production processes. Furthermore, the sausages were characterised with respect to their background microbiota, pH and a_w, as these factors affect the fate of the pathogens. This initial study was not aimed at comparing processing conditions in different commercial meat plants, but to provide a base-line rationale for our subsequent investigation into novel strategies for bacterial pathogen control in fermented sausages.

2. Materials and methods

2.1. Sausages

Investigations were conducted at five commercial meat processing plants located in the northern part of Serbia. Two types of raw, dry, fermented sausages were investigated: a) pork sausages ("Sremska"; produced in plants A, B and C) and beef sausages ("Sudzuk"; produced at plants A, E and D). Sausages were produced using standard raw material composition (Table 1) and standard ripening procedure (Table 1); no meat plant used starter cultures.

2.2. Sampling

At each meat plant and for each of pork or beef sausage type, the sampling was done at following steps: a) chilled meat destined for fermented meat production (meat trimmings); b) chopped meat trimmings; c) complete sausage batter before stuffing in the casings (chopped trimmings + fatty tissue + additives); and d) sausages at three stages of the production process: at the start (day "0"), at mid-process and at the end of the process. For microbiological analysis,

Table 1
Formulations and ripening conditions used for fermented sausages production.

	Pork sausages ("Sremska")						Beef sausages ("Sudzuk")					
	Industry A		Industry B		Industry C		Industry A		Industry D		Industry E	
Formulations												
Meat trimmings	65 kg		65 kg		65 kg		60 kg		60 kg		82 kg	
Fatty tissue	30 kg		30 kg		30 kg		35 kg		35 kg		15 kg	
Spices	Paprika, chillies, coriander, garlic, pepper (2.5 kg in total)		Paprika, chillies, coriander, garlic, pepper (2.5 kg in total)		Paprika, chillies, coriander, garlic, pepper (0.7 kg in total)		Paprika, chillies, onion, pepper, mustard, marjoram, rosemary, basil (2.0 kg in total)		Paprika, chillies, coriander, pepper, turmeric (0.5 kg in total)		Garlic, pepper, celery (0.4 kg in total)	
NaCl–$NaNO_2$ mixture	2.5 kg		2.6 kg		2.5 kg		2.4 kg		2.6 kg		2.2 kg	
Dextrose	0		0.5 kg		0.35 kg		0		0		0.3 kg (+Saccharose 0.2 kg)	
Glucono-δ-lactone (GDL)	1 kg		1 kg		0.6 kg		1.2 kg		1 kg		0	
Ascorbic acid	0.025 kg		0.05 kg		0.015 kg		0.03 kg		0.025 kg		0	
Ripening conditions												
Day	*T*[a]	RH[b]	*T*	RH	*T*	RH	*T*	RH	*T*	RH	*T*	RH
0	24	93	22	98	24	93	24	93	20	90	20	88
1	24	87	18	92	24	87	24	87	18	88	18	84
2	22	84	18	88	22	84	22	84	18	86	18	80
3	19	82	18	86	19	82	19	82	18	84	18	80
4	15	77	17	84	15	77	15	77	18	82	17	78
5	14	75	17	80	14	75	14	75	18	82	17	78
6	14	75	16	70	14	75	14	75	16	68	17	78
7	12	75	16	70	12	75	12	75	16	68	16	75
8	12	75	16	70	12	75	12	75	16	68	15	75
9	12	75	16	70	12	75	12	75	16	68	15	75
10	12	75	16	70	12	75	12	75	16	68	15	75
11	12	75	16	70	12	75	12	75	16	68	15	75
12	12	75	16	70	12	75	12	75	16	68	15	75
13	12	75	16	70	12	75	12	75	16	68	15	75
14	12	75	16	70	12	75	12	75	16	68	15	75

[a] *T* = temperature (°C).
[b] RH = Relative air humidity (%).