Lab on a Chip

Electronic Supplementary Information (ESI)

Fast and on-site animal species identification in processed meat via centrifugal microfluidics and isothermal amplification

Laura Niebling,*[a] Ramona Nitzsche,[c] Thorben Sieksmeyer,[c] Vera Haskamp,[c] Jonas Kissenkötter,[c] Ahmed Abd El Wahed,[d] Thomas Teufel,[e] Herbert Hermann,[f] Nils Paust[ab] and Ana R. Homann*[a]

S1: Preparation of sausages.

Raw fermented sausages (Salami) were prepared either with 50 % frozen and 30 % fresh pork meat (90/10), and 20 % frozen pork fatback (5/95) or with 50 % frozen and 30 % fresh lean beef (95/5), and 20 % frozen beef fat (5/95). Curing salt with 0.5 % sodium nitrite, spice mix "Haussalami Rohwurst Reifekombination CL" (Pacovis, Stetten, Switzerland), and starter culture VBY-81 (SACCO, Cadorago, Italy) were added to the minced meat at 2.8 %, 0.85 %, and 0.02 % respectively. Sausages were processed in a smoking chamber until 30 % drying was reached. The pH value of final salamis was approximately five. Salamis were stored refrigerated at 4 °C for up to two weeks, and then stored frozen.
Cooked sausages (Lyoner) were prepared with 40 % pork meat (S II), 20 % pork belly (S V), 10 % pork cheeks (S VI), 10 % pork fatback (S VIII), 20 % drinking water ice, 1.8 % curing salt, 0.3 % sodium-di-phosphate, 0.6 % spices "Lyoner Gewürz" (Frutarom, Salzburg, Austria). Sausages were filled into sterile casings at calibre 60 mm and cooked at 50 °C for one hour and at 78 °C until 72 °C core temperature was reached. The sausages were cooled at 4 °C for five days, and then stored frozen.
Fine liver sausage was prepared according to the following recipe: 20 % lean pork shoulder (S III), 55 % pork belly (S X) with approximately 50 % fat, 20 % pork liver, and 5 % cooking stock, 1.7 % curing salt (0.5-0.6 %), 0.6 % spice mix "Kalbsleberwurst Moreno Perle" (Frutarom, Salzburg, Austria), 0.3 % dextrose, 0.20 % onion powder, and 0.05 % sodium ascorbate. Pork meat was cooked to 68-70 °C core temperature, minced and mixed with pork liver in a prewarmed cutter (45 %). The meat batter was filled into casings, cooked at 76 °C until 72 °C core temperature was reached, and then cooled at 4 °C for five days. Afterwards, sausages were stored frozen.

S2: Sequences of the developed primer.

Table 1: Sequences of the developed primers to detect the six animal species pork, horse, turkey, sheep, chicken and beef.

Species	Primer	5´-> 3´
Pork	forward	CTACCCTTATCATAACAGTAATGTCCGGAACCAT
	reverse	TGTGGCTGCTTCTGTGGCTCGTG
Horse	forward	CTGCCCCTTGAGAATCAAAATGAACGAAAATC
	reverse	CTAGCCATTGTTGAATTGAGATTAGGCGATTGT
Chicken	forward	ATCCTAGCCTTCTCATCCATCTCCCATTTA
	reverse	GGTTTTAGTTCATGAGATGAGTAGTGTTGACAGT
Turkey	forward	CACCTTTGCATTGTATTCACTAATAACAACAAC
	reverse	GGCCGGCTAGAGATAGGAGTGCAAGTATTATAG
Beef	forward	TAA TAC CTA TTA TCC TAC TAG TCT TCG CAG CC
	reverse	ATT GGA GTA AGT TGA GGT TTT GTA CAT AAT CAG TA
Sheep	forward	CAC AAT AAT ATT CAT CCA CAC AGG ACA
	reverse	GAT CAT GTA ACG AAT AGT GCT ACT GGA ACG

S3: Analysis of sausage samples using a RT-PCR.

As a reference method, the produced sausage samples were tested for animal species using commercially available RT-PCR kits (SureFood®Animal ID series, R-Biopharm AG, Germany). DNA extraction was performed manually using the SureFood®PREP Basic

Kit (R-Biopharm AG, Germany). The limit of detection for the RT-PCR is specified as 0.5 % for pork and 0.1 % for horse, turkey, sheep, chicken and beef. Pork and beef salami as well as cooked pork sausage and liver sausage containing 0 %, 0.1 % and 1 % of horse, pork, turkey, sheep, chicken and beef meat each were analysed. RT-PCR reactions were set up and run according to the manufacturer (SureFood®Animal ID series, R-Biopharm AG, Germany) in duplicates from two separate DNA extractions on a RT-PCR-Cycler (CFX96, Bio-Rad Laboratories GmbH, Feldkirchen, Germany). Signals were evaluated using Bio-Rad CFX Maestro 1.1 (version 4.1.2433.1219, Bio-Rad Laboratories GmbH, Germany) software with "Cq Determination mode" set to "Single Threshold", and "Baseline Setting" set to "Baseline Subtracted Curve Fit". The results of the RT-PCR are shown in Table 1. Regarding pure pork and beef sausages, pork and beef could be detected consistently whereas all other animal species were not detected as expected. 1 % of foreign meat could be detected for all analysed animal species and in all sausages. 0.1 % of horse, pork, turkey and chicken could be detected in all sausages whereas 0.1 % of sheep could only be detected reproducibly in cooked pork sausage. 0.1 % of beef could not be detected in any pork sausage except in one replicate of the cooked pork sausage.

Table 2: Results of the RT-PCR (SureFood®Animal ID, R-Biopharm AG, Germany) following a manual DNA extraction using the SureFood®PREP Basic Kit (R-Biopharm AG, Germany). All runs were carried out in duplicates using samples from pork salami, cooked pork sausage, pork liver sausage and beef salami containing 0 %, 0.1 % and 1 % of horse, pork, turkey, sheep, chicken and beef meat each.

Sausage type	Amount of each foreign animal species [%]	Animal species detected					
		pork	beef	chicken	turkey	horse	sheep
Pork salami	0	2/2	0/2	0/2	0/2	0/2	0/2
	0.1		0/2	2/2	2/2	2/2	0/2
	1		2/2	2/2	2/2	2/2	2/2
Beef salami	0	0/2	2/2	0/2	0/2	0/2	0/2
	0.1	2/2		2/2	2/2	2/2	0/2
	1	2/2		2/2	2/2	2/2	2/2
Cooked pork sausage	0	2/2	0/2	0/2	0/2	0/2	0/2
	0.1		1/2	2/2	2/2	2/2	2/2
	1		2/2	2/2	2/2	2/2	2/2
Pork liver sausage	0	2/2	0/2	0/2	0/2	0/2	0/2
	0.1		0/2	2/2	2/2	2/2	1/2
	1		2/2	2/2	2/2	2/2	2/2