



Figure S1. The main stages in the production of artisanal cheeses. The diagram contains the main stages of the artisanal cheeses production and it was compiled based on the information obtained from the producers.

MALDI TOF MS identification

Table 1. Identification of the *L. monocytogenes* isolates by MALDI TOF MS.

Analyte Name	Organism (best match)	Score Value	Organism (second best match)	Score Value
T 23 2(++) (A)	Listeria monocytogenes	2.02	Listeria monocytogenes	2.002
T 10 1(+)(B)	Listeria monocytogenes	1.996	Listeria innocua	1.996
T 11 1(+)(B)	Listeria monocytogenes	1.96	Listeria monocytogenes	1.931
T 13B 2(++) (A)	Listeria monocytogenes	2.054	Listeria monocytogenes	1.994
T 2A 1(++) (A)	Listeria monocytogenes	2.007	Listeria innocua	1.993
T 2B 2(+)(B)	Listeria monocytogenes	1.997	Listeria monocytogenes	1.991
T 6A 1(++) (B)	Listeria monocytogenes	2.101	Listeria innocua	2.023
T 16 3(++) (A)	Listeria monocytogenes	2.005	Listeria monocytogenes	1.951
T 19 2(+)(B)	Listeria monocytogenes	1.986	Listeria monocytogenes	1.955
T 4 1(++) (B)	Listeria monocytogenes	2.239	Listeria innocua	2.203
T 5 1(++) (B)	Listeria monocytogenes	2.112	Listeria monocytogenes	2.063
T 10 2(++) (B)	Listeria monocytogenes	2.182	Listeria monocytogenes	2.139
T 10 3(++) (B)	Listeria monocytogenes	2.106	Listeria monocytogenes	2.087
T 11 2(+)(B)	Listeria monocytogenes	1.997	Listeria monocytogenes	1.966
T 11 3(+)(B)	Listeria monocytogenes	1.997	Listeria monocytogenes	1.994
T 12 1(+)(B)	Listeria monocytogenes	1.921	Listeria monocytogenes	1.88
T 12 2(+)(B)	Listeria monocytogenes	1.901	Listeria welshimeri	1.834
T 12 3(+)(B)	Listeria monocytogenes	1.929	Listeria monocytogenes	1.87
T 13A 1(++) (A)	Listeria monocytogenes	2.023	Listeria monocytogenes	2.022
T 13A 2(++) (A)	Listeria monocytogenes	2.048	Listeria monocytogenes	2.023
T 13A 3(++) (A)	Listeria monocytogenes	2.024	Listeria monocytogenes	1.992
T 13B 1(+)(B)	Listeria monocytogenes	1.903	Listeria monocytogenes	1.862
T 13B 3(++) (A)	Listeria monocytogenes	2.112	Listeria monocytogenes	2.03
T 13C 1(++) (B)	Listeria monocytogenes	2.065	Listeria monocytogenes	2.049
T 13C 2(+)(B)	Listeria monocytogenes	1.991	Listeria monocytogenes	1.935
T 13C 3(++) (A)	Listeria monocytogenes	2.021	Listeria monocytogenes	1.996
T 14A 1(+++) (B)	Listeria monocytogenes	2.368	Listeria monocytogenes	2.317
T 14A 2(+++) (B)	Listeria monocytogenes	2.306	Listeria monocytogenes	2.276
T 14A 3(+++) (B)	Listeria monocytogenes	2.326	Listeria monocytogenes	2.272
T 14B 1(++) (A)	Listeria monocytogenes	2.013	Listeria monocytogenes	1.978
T 14B 2(++) (A)	Listeria monocytogenes	2.106	Listeria monocytogenes	2.017
T 14B 3(++) (A)	Listeria monocytogenes	2.106	Listeria monocytogenes	2.033
T 15 1(+)(B)	Listeria monocytogenes	1.812	Listeria monocytogenes	1.777
T 15 2(+)(B)	Listeria monocytogenes	1.951	Listeria monocytogenes	1.935
T 15 3(++) (A)	Listeria monocytogenes	2.048	Listeria monocytogenes	1.979
T 16 1(++) (A)	Listeria monocytogenes	2.036	Listeria monocytogenes	1.958
T 16 2(+)(B)	Listeria monocytogenes	1.803	Listeria monocytogenes	1.771
T 17 1(++) (B)	Listeria monocytogenes	2.05	Listeria innocua	2.048
T 17 2(++) (B)	Listeria monocytogenes	2.086	Listeria monocytogenes	2.075
T 17 3(++) (B)	Listeria monocytogenes	2.08	Listeria innocua	2.046
T 18 1(++) (B)	Listeria monocytogenes	2.268	Listeria monocytogenes	2.15
T 18 2(++) (B)	Listeria monocytogenes	2.262	Listeria monocytogenes	2.131
T 18 3(++) (B)	Listeria monocytogenes	2.249	Listeria monocytogenes	2.152

T 19 1(+) (B)	Listeria monocytogenes	1.976	Listeria ivanovii	1.967
T 19 3(+) (B)	Listeria monocytogenes	1.988	Listeria ivanovii	1.98
T 1A 1(++) (B)	Listeria monocytogenes	2.272	Listeria monocytogenes	2.213
T 1A 2(++) (B)	Listeria monocytogenes	2.258	Listeria monocytogenes	2.229
T 1A 3(++) (B)	Listeria monocytogenes	2.041	Listeria monocytogenes	2.026
T 1B 1(++) (A)	Listeria monocytogenes	2.028	Listeria monocytogenes	2.027
T 1B 2(++) (B)	Listeria monocytogenes	2.045	Listeria monocytogenes	2.019
T 1B 3(++) (B)	Listeria monocytogenes	2.055	Listeria innocua	2.05
T 1C 1(++) (B)	Listeria monocytogenes	2.169	Listeria innocua	2.154
T 1C 2(++) (B)	Listeria monocytogenes	2.193	Listeria innocua	2.139
T 1C 3(++) (B)	Listeria monocytogenes	2.087	Listeria monocytogenes	2.025
T 20 1(+) (B)	Listeria monocytogenes	1.955	Listeria monocytogenes	1.917
T 20 2(+) (B)	Listeria monocytogenes	1.922	Listeria monocytogenes	1.895
T 20 3(+) (B)	Listeria monocytogenes	1.936	Listeria monocytogenes	1.934
T 21 1(++) (B)	Listeria monocytogenes	2.116	Listeria monocytogenes	2.074
T 21 2(++) (A)	Listeria monocytogenes	2.052	Listeria monocytogenes	1.998
T 21 3(++) (B)	Listeria monocytogenes	2.153	Listeria monocytogenes	2.067
T 22 1(++) (B)	Listeria monocytogenes	2.099	Listeria monocytogenes	2.055
T 22 2(++) (A)	Listeria monocytogenes	2.101	Listeria monocytogenes	2.037
T 22 3(++) (A)	Listeria monocytogenes	2.045	Listeria monocytogenes	1.956
T 23 1(+) (B)	Listeria monocytogenes	1.905	Listeria monocytogenes	1.903
T 23 3(+) (B)	Listeria monocytogenes	1.998	Listeria welshimeri	1.943
T 2A 2(+) (B)	Listeria monocytogenes	1.915	Listeria monocytogenes	1.89
T 2A 3(++) (B)	Listeria monocytogenes	2.141	Listeria monocytogenes	2.133
T 2B 1(+) (B)	Listeria monocytogenes	1.935	Listeria monocytogenes	1.923
T 2B 3(++) (A)	Listeria monocytogenes	2.033	Listeria monocytogenes	1.985
T 2C 1(++) (A)	Listeria monocytogenes	2.061	Listeria monocytogenes	2.014
T 2C 2(++) (A)	Listeria monocytogenes	2.033	Listeria monocytogenes	1.973
T 2C 3(++) (B)	Listeria monocytogenes	2.152	Listeria monocytogenes	2.133
T 3 1(++) (B)	Listeria monocytogenes	2.28	Listeria innocua	2.28
T 3 2(++) (B)	Listeria monocytogenes	2.048	Listeria ivanovii	2.047
T 3 3(+) (B)	Listeria monocytogenes	1.997	Listeria innocua	1.961
T 4 2(++) (B)	Listeria monocytogenes	2.278	Listeria monocytogenes	2.247
T 4 3(++) (B)	Listeria monocytogenes	2.29	Listeria monocytogenes	2.225
T 5 2(++) (B)	Listeria monocytogenes	2.052	Listeria monocytogenes	2.03
T 5 3(++) (B)	Listeria monocytogenes	2.191	Listeria innocua	2.107
T 6A 2(++) (B)	Listeria monocytogenes	2.134	Listeria monocytogenes	2.091
T 6A 3(++) (B)	Listeria monocytogenes	2.137	Listeria ivanovii	2.105
T 6B 1(+) (B)	Listeria monocytogenes	1.946	Listeria welshimeri	1.865
T 6B 2(++) (A)	Listeria monocytogenes	2.043	Listeria monocytogenes	1.99
T 6B 3(+) (B)	Listeria monocytogenes	1.947	Listeria innocua	1.93
T 7A 1(++) (B)	Listeria monocytogenes	2.122	Listeria monocytogenes	2.093
T 7A 2(++) (B)	Listeria monocytogenes	2.042	Listeria monocytogenes	2.027
T 7A 3(++) (A)	Listeria monocytogenes	2.113	Listeria monocytogenes	2.062
T 7B 1(++) (A)	Listeria monocytogenes	2.065	Listeria monocytogenes	2.025
T 7B 2(++) (A)	Listeria monocytogenes	2.095	Listeria monocytogenes	2.077
T 7B 3(++) (A)	Listeria monocytogenes	2.031	Listeria monocytogenes	2

T 8 1 (++) (A)	Listeria monocytogenes	2.027	Listeria monocytogenes	2.009
T 8 2 (++) (B)	Listeria monocytogenes	2.234	Listeria monocytogenes	2.2
T 8 3 (++) (B)	Listeria monocytogenes	2.121	Listeria monocytogenes	2.103
T 9 1 (++) (B)	Listeria monocytogenes	2.144	Listeria monocytogenes	2.112
T 9 2 (++) (B)	Listeria monocytogenes	2.164	Listeria monocytogenes	2.136
T 9 3 (++) (B)	Listeria monocytogenes	2.127	Listeria monocytogenes	2.12

Table 2. Meaning of score values.

Range	Description	Symbols	Color
2.300 ... 3.000	highly probable species identification	(+++)	green
2.000 ... 2.299	secure genus identification, probable species identification	(++)	green
1.700 ... 1.999	probable genus identification	(+)	yellow
0.000 ... 1.699	not reliable identification	(-)	red

Molecular serotyping of *L. monocytogenes* by multiplex PCR

The Multiplex PCR for identification of the main *L. monocytogenes* serogroups was performed as described previously (Doumith et al., 2004; Wiczorek et al., 2012). The gene amplification was carried out in a thermal cycler (Biometra, Göttingen, Germany) under the following conditions: initial DNA denaturation at 94°C for 5 min, followed by 30 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min. The final cycle was performed at 55°C for 2 min and 72°C for 5 min. The protocol developed by Doumith et al. (2014) was used to determine the molecular serogroup affiliation of the *L. monocytogenes* tested. This method facilitates assigning isolates to one of four molecular serogroups (IIa, IIb, IIc, or IVb) by the presence of the genes listed in Table S3.

Table 3. Presence of lmo0737, lmo1118, ORF2819, ORF2810 and prs in *L. monocytogenes* molecular serogroups.

Serogroups (serotypes)	Presence of gen				
	lmo118	lmo0737	ORF2110	ORF2819	prs
IIa (1/2a, 3a)		▪			▪
IIb (1/2b, 3b)				▪	▪
IIc (1/2c, 3c)	▪	▪			▪
IVb (4b,4d,4e)•			▪	▪	▪

The reaction mixture with the constituents shown in Table S4 was prepared for the multiplex PCR, the primers for the reaction were those stated in Table S5 and the parameters of the PCR are set out in Table S6.

Table 4. Constituents of the reaction mixture in the multiplex PCR assay for *L. monocytogenes* molecular serogroup identification.

Reaction mixture PCR (starting concentration)	Quantity (µl)
water	22
bufo PCR (10x)	5
MgCl ₂ (25 mM)	6

dNTP (2 mM)	5
Starters (10 mM):	
lmo1118F	0,5
lmo1118R	0,5
lmo0737F	0,5
lmo0737R	0,5
ORF2110F	0,5
ORF2110R	0,5
ORF2819F	0,5
ORF2819R	0,5
prsF	0,5
prsR	0,5
Polymerase (1 U/μl)	2
DNA	5
Total	50

Table 5. Primers used in the multiplex PCR assay for *L. monocytogenes* molecular serogroup identification.

Amplified gen	Starter name	Sequence (5' → 3')	Product size (pz)
lmo1118	lmo1118F	AGGGGTCTTAAATCCTGGAA	906
	lmo1118R	CGGCTTGTTCCGCATACTTA	
lmo0737	lmo0737F	AGGGCTTCAAGGACTTACCC	691
	lmo0737R	ACGATTTCTGCTTGCCATTC	
ORF2110	ORF2110F	AGTGGACAATTGATTGGTGAA	597
	ORF2110R	CATCCATCCCTTACTTTGGAC	
ORF2819	ORF2819F	AGCAAAATGCCAAAACCTCGT	471
	ORF2819R	CATCACTAAAGCCTCCCATG	
prs	prsR	GCTGAAGAGATTGCGAAAGAAG	370
	prsF	CAAAGAAACCTTGGATTGCGG	

Table 6. Multiplex PCR assay parameters for *L. monocytogenes* molecular serogroup identification.

Temperature (°C)	Number of cycles	Time (min)
94,0	1	5
55,0	30	1
72,0		2
94,0		1
55,0	1	2
72,0	1	5
15,0	∞	

After amplification, 5μl of 10× DNA gel loading buffer was added to each sample and the samples were applied to 1.5% agarose gel and electrophoresed for 40 min at 100V. The results were viewed with a GelDoc 2000 transilluminator (Bio-Rad, Hercules, CA, USA). The GeneRuler 100bp DNA Ladder (Thermo Fisher Scientific, Waltham, MA, USA) was used as the molecular weight standard.

References

1. Doumith, M.; Buchrieser, C.; Glaser, P.; Jacquet, C.; Martin, P. Differentiation of the major *Listeria monocytogenes* serovars by multiplex PCR. *J. Clin. Microbiol.* **2004**, *42*, 3819–3822. doi:10.1128/JCM.42.8.3819-3822.2004

2. Wieczorek, K.; Dmowska, K.; Osek, J. Characterization and antimicrobial resistance of *Listeria monocytogenes* isolated from retail beef meat in Poland. *Foodborne Pathog. Dis.* **2012**, *9*, 681–685. doi:10.1089/fpd.2012.1137