METHODS FOR LISTERIA INOCULATION; VALIDATION OF A SURFACE AND A MIXING METHOD

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Background

The development of *Listeria monocytogenes* growth models starts with the initial recovery, proceeds with determining growth and finishes with determining the influence of different factors on growth. If there is no prevalence of Listeria on the product, it can be inoculated with this pathogen. For a comparison of growth between different products it is important to start with consistent low inoculation levels because (i) microbial growth can be dependent on initial numbers and (ii) in practice Listeria contamination will start at low numbers. To study Listeria growth with, for instance, different packaging techniques (Cagri et al. 2002), surface treatments (Naidu et al. 2003), or ingredient compositions (Sabia et al. 2003) different inoculation techniques are used. In the present study, two simple, safe and cost effective methods are compared. The first method consists of inoculating the surface using a horizontal glass plate (HGP). The second method blends the Listeria inoculum into the meat sample (BM) while the meat is chopped.

Objectives

This study was conducted to compare two inoculation methods for *Listeria monocytogenes* on meat; one by surface inoculation of an intact cooked slice of beef and the other by mixing the inoculum while chopping the cooked beef.

Materials and methods

Sample preparation: Beef longissimus steaks (2.5 cm thick) were vacuum packed, heated in a water bath at 70°C for 60 minutes and chilled on ice water. Products were stored for 1 or 2 days at 4°C until used for one of two treatments.
Inoculation: The treatment with surface inoculation (HGP) was carried out using a glass plate (30x30 cm) with a welded metal rim (height = 1 cm) put horizontally using a levelling table. Thirty mL of inoculation suspension was pipetted on the glass plate area, making sure it covered the whole area. The inoculum suspension contained 140.000 CFU/mL *Listeria monocytogenes* ATCC7644 (fresh overnight culture in Brain Heart Infusion broth at 37°C), and a surfactant (Tween 80, 0.01%) was added to facilitate even spreading on the glass plate. The resulting inoculation surface contained 3.67 log CFU/cm^2. One side of the cooked steaks was gently pressed on the glass plate, making sure there was good surface contact. The steaks were again vacuum packed. At the start of the experiment 25 steaks were inoculated, 10 were used at day 0 and after storage at 4°C for 5, 8, 12, 15 and 25 days, 3 steaks were sampled per day.
For the second treatment (BM), 2.0 kg of the cooked meat was chopped until course, using a commercial chopper (RK 30 SL, Kilia, Kiel, Germany). Subsequently, 20 mL of the inoculum containing 10.000 CFU/mL *Listeria monocytogenes* was evenly poured in the chopper. The meat was chopped until very fine. The chopped meat was vacuum packed in portions of 200 g. Ten portions were sampled on the day of inoculation. The others were stored at 4°C for 5, 8, 12, 15 and 25 days. Two portions were sampled per day.
Microbiological analyses: Samples for microbiological analyses were taken with a sterile cork borer, diluted (1:10 w/w) and stomachered in Peptone Physiological Salt solution (2 min). *Listeria monocytogenes* colonies were counted on Rapid L. mono agar (BioRad) after 24 h at 37°C. Total Aerobic Psychrophylic bacteria were counted on Plate Count Agar (Merck) after 3 days at 20°C. BM samples were analysed in duplicate and HGP samples were analysed in triplicate to compensate for possible uneven distribution.
Data was analysed using Microsoft Excel and a two sided T-test was performed.

Results and discussion

The results from the two different inoculation methods (Table 1) were significantly different for recovery percentage ($p<0.05$). For HGP the recovery can be calculated per cm^2, based on the surface of the cork borer (5 cm^2). The HGP method has on average a recovery percentage of 21.8 (SD 4.8) and an inoculation level of 1.67 log CFU listeria/g (SD 0.07) versus a recovery percentage of 15.6 (SD 6.7) and an inoculation level of 1.15 log CFU listeria/g (SD 0.19) for the BM method. Inoculation levels are not statistically compared because the initial loads are not comparable.
The low recovery for BM is possibly explained by a by a damaging effect of the shear forces which occur by chopping at high speed (4000 rpm). The recovery percentage for the HGP method can be explained by the efficiency of transfer of microorganisms from the glass surface to the meat surface. The results of the total aerobic psychrophyles count confirmed the results found with the Rapid L. mono agar, indicating that the selectivity of Rapid L. mono agar had no influence on the results (data not shown).
The growth of Listeria in the vacuum packed beef occurred approximately at the same rate for both inoculation methods (Figure 1). The slope of the growth is 0.02 Log CFU Lm/day for both inoculation methods (0.0219 for HGP and 0.0196 for BM). This rate of growth is low for *Listeria monocytogenes* in cooked beef product compared to the ARS ERRC Pathogen Modelling Program (http://www.arserrc.gov/mfs/PATHOGEN.HTM). The ARS ERRC Pathogen Modelling Program has been developed with broth models. A study form Duffy et al. (1994) has modelled the growth of Listeria in cooked beef. In this study relative long lag phases were determined and growth is modelled over a 35-day period. The products prepared by Duffy et al. contained 2% added water and gelatine for better slicing quality. In this study water loss from the cooking process was not used for the inoculation methods. The slower growth of Listeria monocytogenes in this study can be explained by a lower moisture content in the samples.

Conclusions

The method of surface inoculation using a horizontal glass plate (HGP) resulted in a significantly higher recovery of 21.8% versus 15.6% ($p<0.05$) for the method whereby the meat was chopped (BM) and at the same time the Listeria inoculum was blended in.
The low percentage for the BM method can be explained by a damaging effect of the shear force, which occurs during the chopping process. An inoculation method should not deactivate microorganisms, unless aimed for, because it can have an effect on the growth characteristics. Therefore the BM method is less suitable. An effect of the inoculation method on growth rate was not observed in this study.
For the HGP method there were no indications of a damaging effect on *Listeria monocytogenes*. In addition, the HGP method resulted in the lowest standard deviation in inoculation levels. For easy inoculation with *Listeria monocytogenes* the HGP method is therefore preferred.

References

Cagri, A., Ustunol, Z., Ryser, E.T., (2002) Inhibition of three pathogens on bologna and summer sausage using antimicrobial edible film. Journal of Food Science **67**: 6, 2317-2324

Duffy, L.L., Vanderlinde, P.B., Grau, F.H., (1994) Growth of *Listeria monocytogenes* on vacuum-packed cooked meats: effects of pH, aw, nitrite and ascorbate. International Journal of Food Microbiology **23**: 377-390

Naidu, A.S., (2002) Activated lactoferrin - A new approach to meat safety. Food technology **56**: 40-45

Sabia, C., Niederhausern S. de, Messi, P., Manicardi, G., Bondi, M., (2003) Bacteriocin-producing Enterococcus casseliflavus IM 416K1, a natural antagonist for control of Listeria monocytogenes in Italian sausages ("cacciatore), International Journal of Food Microbiology **87**: 1-2, 173-179

Eastern Region Research Center (2004) Pathogen Modelling Program Version 7.0, http://www.arserrc.gov/mfs/PATHOGEN.HTM)

Table 1: *Listeria monocytogenes* counts from two different inoculation methods, HGP and BM

Method	Horizontal Glass Plate			Blending		
	Log CFU/g			Log CFU/g		
	AV	SD	Recovery (%)	AV	SD	Recovery (%)
1	1.68	0.07	25.6	1.40	0.17	25.2
2	1.59	0.18	19.6	1.30	0.00	20.0
3	1.66	0.08	16.7	1.26	0.24	18.2
4	1.70	0.03	26.2	0.80	0.17	6.3
5	1.70	0.13	24.6	0.90	0.17	7.9
6	1.79	0.04	27.1	1.23	0.50	17.1
7	1.58	0.09	15.6	1.36	0.10	22.9
8	1.61	0.09	16.1	1.00	0.30	10.0
9	2.07[1]	0.21[1]	[1]	1.10	0.35	12.6
10	1.74	0.17	24.9	1.10	0.35	12.6
AV	1.67	0.07	21.8	1.15	0.19	15.6

1 outlying observation based on Grubbs equation

Figure 1: Growth of inoculated *Listeria monocytogenes* in vacuum packed cooked beef