Comparison of the effectiveness of post-lethality treatments and antimicrobial agents to reduce the risk of *Listeria monocytogenes* in RTE meat and poultry products

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Introduction

Listeria monocytogenes can be present in ready-to-eat foods due to post-processing contamination. Different methods can be adopted to reduce the risk of illness or death from *L. monocytogenes* in these products. Post-lethality treatments, such as High Pressure Processing, post-pasteurization or bactericidal surface treatments, and / or antimicrobial agents, such as lactates and diacetates, can be applied to reduce the presence and /or limit the outgrowth of *L. monocytogenes* on the meat product.

High Pressure Processing kills various microorganisms in food products by non-thermal inactivation. Several studies have reported the recovery of damaged microorganisms, indicating the potential for outgrowth during shelf life which could lead to an increased risk of food poisoning (Bozoglu et al., 2004; Bull et al., 2005). Alternative techniques like post-pasteurization and bactericidal surface treatment also do not inactivate all organisms.

Antimicrobial agents such as lactates and diacetates can control the outgrowth of *L. monocytogenes* during the shelf life of ready-to-eat meat products but do not reduce the initial count.

This study compares the effectiveness of these methods, both alone and in combination, to reduce the risk of *L. monocytogenes*.

Materials & methods

Inoculation study: The inoculation study compares the efficacy of a bactericidal surface treatment with lauric arginate, with the addition of lactate and diacetate as ingredients. The combination of both methods is also included. Table 1 gives an overview of the different variants. Pork ham samples containing 0% (control) or 2.5% PURASAL *Opti.Form* PD4 were surface inoculated with a 4 strain cocktail of *L. monocytogenes* at approximately 3 log10 CFU/g. After 30 min., the appropriate samples were surface treated with 0.07% Protect-M using the SLIC method (Luchansky et al, 2005). Subsequently, all samples were stored at 4°C and analyzed for *L. monocytogenes* during 72 days.

Table 1. The test variants included in the inoculation study

No.	Ingredient	Surface treatment
1	- (Control)	- (Control)
2	3% PURASAL *Opti.Form* PD4 (1.7% potassium lactate, 0.12% sodium diacetate)	0.07% Protect-M (70 ppm lauric arginate)
3	-	0.07% Protect-M (70 ppm lauric arginate)

Inoculum preparation: Cultures of *L. monocytogenes* (culture collection number LMG 1678, NCTC 12480, NCIMB 1344, LMG 2319) were started from plate and incubated two nights at 12ºC in screw-capped tubes (100 x 16 mm) containing 10 ml brain heart infusion broth (Oxoid CM0225, Basingstoke, UK).

Microbial analysis: At appropriate time intervals, samples of pork ham of each batch were taken in duplicate for microbiological analyses. A sample bag was opened and to this was added 2 times the net weight sterile dilution fluid (8.5 % (w/w) NaCl and 0.1 % (w/v) bacteriological peptone. The mix was homogenized for 1 min. in a Stomacher 400 lab blender (Seward Medical, London, England). 50 µl of the homogenate was subsequently plated on Palcam agar (Oxoid CM877 with selective supplement SR0150) using an Eddyjet type 1.23 spiral plater (IUL Instruments, Barcelona, Spain). Plates were incubated for 48 hours at 30 ºC. Homogenization and subsequent plating was carried out in triplicate.

Mathematical simulations: PURAC's *Opti.Form* Listeria Control Model 2007 (www.purac.com) was used to simulate the effect of post-lethality treatments alone or in combination with lactates and diacetates on the outgrowth of *L. monocytogenes*. Simulations were carried out for a cured pork ham containing 2% salt,

74% moisture, pH 6.2 that is contaminated with 1 log10 CFU/g. Different bactericidal and bacteriostatic treatments were simulated.

Results and discussion

Inoculation study: The results of the inoculation study are presented in Figure 1. The surface treatment with lauric arginate results in an immediate reduction of about 1 log10 CFU/g, both for the sample without additives and the sample containing lactate and diacetate. Outgrowth of surviving cells starts immediately for the sample without additives, at the same growth rate as the control. The surviving cells are apparently not damaged by the lauric arginate treatment. Compared to the control, the time to 1 log10 CFU/g outgrowth is extended with only 7 days.

The untreated sample containing lactate and diacetate shows no outgrowth for 72 days, demonstrating that the efficacy of additives like lactate and diacetate outperforms that of a mild post-lethality treatment that has no residual protective effect.

The combination of a surface treatment with lauric arginate and the addition of lactate and diacetate as ingredients gives a reduction of 1 log10 CFU/g and controls Listeria outgrowth during at least 72 days.

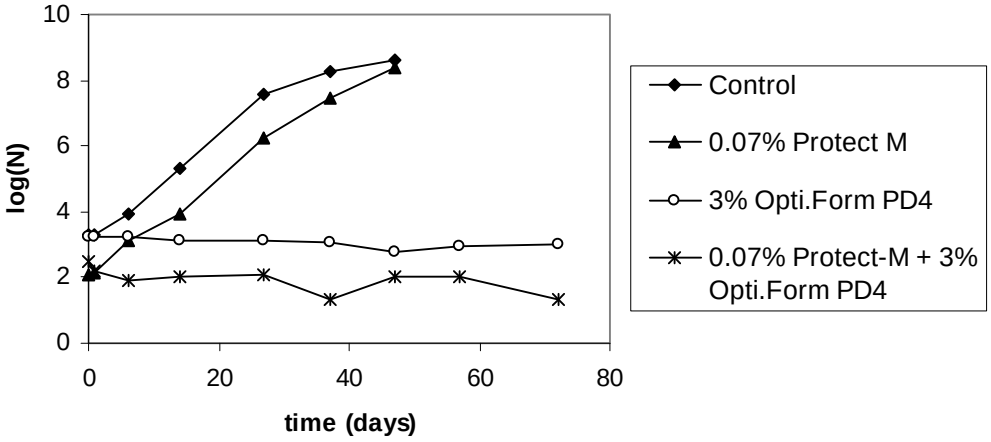

Figure 1. Survival and outgrowth of *L. monocytogenes* at 4°C on pork ham surface treated with Protect-M and/or containing PURASAL *Opti.Form* PD4.

Mathematical simulations

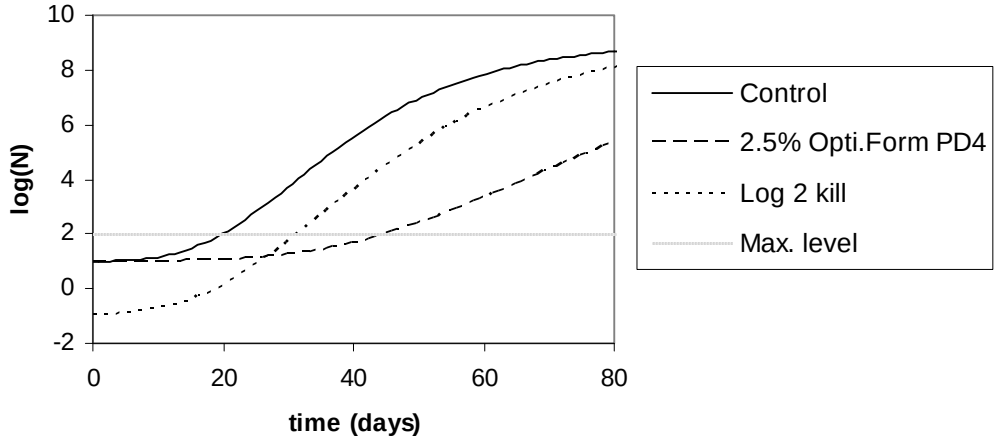

Figure 2. Mathematical simulations of the outgrowth of *L. monocytogenes* in cured pork ham for a bacteriostatic treatment with 2.5% PURASAL *Opti.Form* PD4 and a bactericidal treatment giving a 2 log10 CFU/g kill compared to an untreated control.

Figure 2 compares the efficacy of a bacteriostatic treatment with 2.5% PURASAL *Opti.Form* PD4 and a bactericidal treatment giving a 2 log10 CFU/g kill. The treatment with *Opti.Form* extends the time to 1 log10

outgrowth with 26 days; for the bactericidal treatment, this is only 12 days. To obtain a shelf life extension of 26 days using a bactericidal treatment, a reduction of 5 log10 CFU/g is required (Figure 3).

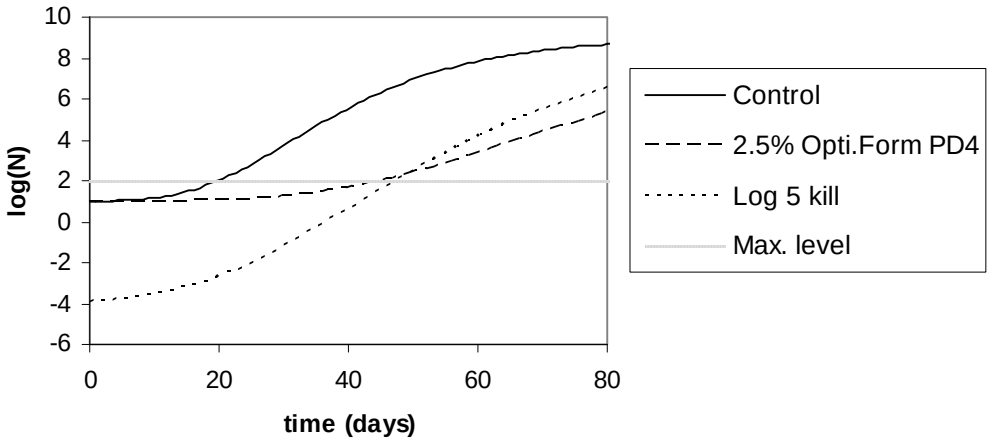

Figure 3. Mathematical simulations of the outgrowth of *L. monocytogenes* in cured pork ham for a bacteriostatic treatment with 2.5% PURASAL *Opti.Form* PD4 and a bactericidal treatment giving a 5 log10 CFU/g kill compared to an untreated control.

Conclusions

The inoculation study and the mathematical simulations both demonstrate that a bactericidal treatment by itself is not sufficient to reduce the risk of illness or death from *L. monocytogenes* in meat products. This is mainly due to the absence of residual protection after the treatment. It is recommended to combine post-lethality treatment with an antimicrobial to ensure residual protection in the final product against the outgrowth of surviving microorganisms and contamination after opening. The combination of a surface treatment using Protect-M with the use of PURASAL *Opti.Form* PD4 is a very effective way to minimize the risk of *L. monocytogenes*.

References

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