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# HEAT INACTIVATION OF *LISTERIA MONOCYTOGENES* IN SOUS VIDE COOKED BEEF PREPARED AT RISING TEMPERATURES

TINA BECK HANSEN, GITTE BRANDT & SUSANNE KNØCHEL
LMC-Centre for Advanced Food Studies, The Royal Veterinary and Agricultural University, Howitzvej 13, 3.sal, DK-2000 Frederiksberg, Denmark.

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## Background

Sous vide cooked foods are produced in increasing amounts in the catering and food processing industry. It is estimated that in the region of France, Belgium, The Netherlands and Switzerland the annual growth of sous vide cooked meat is about 12% (Pralus, 1993). Sous vide technology is a process characterized by vacuum packaging in heat stable, high-barrier bags followed by pasteurization and quick cooling before refrigerated storage below 3°C. Sous vide cooked foods usually undergo very gentle heat treatments and some concern has been expressed about the microbiological risks involved (Church & Parsons, 1993).

One of the microorganisms of prime interest with regard to safety of sous vide cooked beef is *Listeria monocytogenes* due to its widespread occurence, psychrotrophic nature and relatively high heat resistance. Heating at slowly rising temperatures is suspected to enhance heat resistance of *Listeria monocytogenes* (Quintavalla & Campanini, 1991; Kim *et al.*, 1994; Stephens *et al.*, 1994) and since anaerobic environment has been shown to facilitate resuscitation of heat injured cells of this microorganism (Knabel *et al.*, 1990; Kim *et al.*, 1994) concern may arise about the possibility of *L. monocytogenes* to survive the heat treatment employed in sous vide cooked products.

## Objective

The purpose of the present study was to determine the effect of slow and fast heating on heat inactivation of *L. monocytogenes* in sous vide cooked beef prepared at mild processing temperatures.

## Materials and methods

*Sample preparation. Listeria monocytogenes* (serotype 1) isolated from pasteurized cured ham was obtained from The Danish Veterinary Service, Food Control Laboratory, Denmark. The strain was maintained as a frozen suspension at -80°C. The culture was grown to late exponential phase (17 h) at 30°C in shaking culture in tryptic phosphate broth (TPB; Conner *et al.*, 1986). Cells were harvested by centrifugation and resuspended in fresh TPB.

Fresh beef (*M. semitendinosus)* was deep fried in an electric deep frier (TEFAL Super-Friteuse) for ½-1 min to destroy the surface microflora. The cooked exterior was aseptically removed to a depth of ½-1 cm. Subsequently, the meat was aseptically cut into smaller blocks and passed through a 0.4 cm holeplate in a sterilized food processor (Kenwood chef excel). With a flamed spoon portions of 10±0.2 g meat mince were weighed into individual 8x5 $cm^2$ vacuum pouches (Cryovac BT300 bags) pressed to the bottom and shaped into a meat block. pH of the raw material for each trial was measured using a direct insertion probe electrode (LOT406-M3-S7/25 INGOLD) and a Portamess 751 Calimatic pH-meter (Knick). The average pH of the beef was 5.64 (S.D.= 0.08).

*L. monocytogenes* inocula of 0.2 ml was injected with syringe and needle into the geometric centre of the meat samples. Subsequently, the samples were evacuated in a Komet model X 200 and heat sealed.

*Heating and cooling procedures.* The meat samples were placed in a rack with no contact between the samples. To achieve fast heating samples were immersed in circulating water equilibrated to test temperatures of 56, 60 and 64°C, respectively. To achieve slow heating samples were immersed in water of 18-20°C and heated at linear rates of 0.3 or 0.6°C $min^{-1}$ (programmable thermostat: Heto Centigrade) up to the test temperatures. After reaching the terminal temperature the samples were maintained at this temperature. At different time intervals duplicate samples were removed from the hot water bath and placed in a water bath of 10°C until analyzed (minimum 10 min, maximum 2 h).

*Temperature monitoring.* During heating core temperatures in two samples were continuously monitored using thermocouples and data logger (Grant 1200 series Squirrel meter/logger). In each trial the recorded time and temperatures were converted to pasteurization values, as described by Shapton & Shapton (1991), and used as time scale of the survivor curves.

*Enumeration.* The bags were aseptically opened and the samples (meat and juice) diluted ten-fold in University of Vermont broth (without selective agents) and homogenized. Decimal dilutions in physiological saline with 0.1% peptone were surface plated on tryptic soy agar + 0.6% yeast extract + 0.1% sodium pyruvate. When low counts (< 100 CFU $g^{-1}$) were anticipated, a five tubes Most Probable Number (MPN) analysis was performed in tryptic soy broth + 0.6% yeast extract + 0.1% sodium pyruvate. Plates and tubes were incubated at 30°C for 5 d. Material from positive MPN-tubes were streaked onto *Listeria* selective agar Oxford formulation and incubated for 24 h at 30°C for verification.

*Data analysis.* A full factorial design of heating temperature (56, 60 and 64°C) and type of rising period (>10, 0.6 and 0.3°C $min^{-1}$) was conducted with duplicate trials. Linearly rising heating of 0.1°C $min^{-1}$ and two examples of heat shock (46°C in 30 min, 48°C i 120 min) were performed as single trials at 60°C. The different types of rising periods were defined as pretreatments and the heating regimes were compared using *decimal reduction times* (D-values) calculated from the survivor curves starting when the test temperature was reached.

$Log_{10}$ to the D-values were used as response variable in a covariate analysis with pH of the raw material as covariate and test temperature as well as type of rising period as fixed effects.

## Results and discussion

Previous work in pork emulsion containing curing salts has demonstrated that heating at rising temperatures (0.5°C $min^{-1}$) increases the heat resistance of *L. monocytogenes* compared to instantaneous heating by almost two-fold (Quintavalla & Campanini, 1991). Heating grounded pork at 1.3°C $min^{-1}$ compared to 8.0°C $min^{-1}$ also increased the D-value by almost two-fold (Kim *et al.*, 1994). Stephens *et al.* (1994) recently showed that a heating rate of ≤0.7°C $min^{-1}$ induced maximum heat resistance in *L. monocytogenes* when heated in a broth (TPB) model system. Heating rates faster than 5°C $min^{-1}$ did not induce increase in heat resistance at all (Stephens *et al.*, 1994). In the present study (Table 1), however, no statistically significant difference (P=0.696) was observed between the following type of rising periods; >10°C $min^{-1}$, 0.6°C $min^{-1}$ and 0.3°C $min^{-1}$. Neither for cells heated for 0.1°C $min^{-1}$ nor for heat shocked cells significant increase in D-values of practical importance was observed (Table 1). These results were not in agreement with any published literature on the subject and it was suspected that the low pH of beef (5.64) could be the reason for this.

**Table 1.** Average D-values (min) for *Listeria monocytogenes* heated in vacuum-packed minced beef under different heating regimes

| | Type of rising period | | | | | |
|---|---|---|---|---|---|---|
| Target temperature (°C) of heat treatment | Rapid rise, >10°C $min^{-1}$ | Linear rise (°C $min^{-1}$) | | | Heat shock (°C/min) | |
| | | 0.6 | 0.3 | 0.1 | 46/30 | 48/120 |
| 56 | 26.8 | 27.5 | 29.2 | NT | NT | NT |
| 60 | 6.7 | 6.4 | 7.1 | 8.1[a] | 7.2[a] | 8.4[a] |
| 64 | 1.4 | 1.6 | 1.7 | NT | NT | NT |
| z-value (°C) | 6.9 | 6.5 | 6.6 | | | |

NT: Not tested
a: Only one trial

Further supporting experiments from our laboratory (data not shown) showed that the effect of heat shock (46°C in 30 min) on the heat resistance of *L. monocytogenes* heated in beef with pH of 5.4 and 5.8 was not statistically significant (P=0.310 and P=0.069, respectively), while heat shocking cells in beef with pH of 6.2 resulted in a statistically significant (P=0.003) increase in D-value of more than two-fold.

## Conclusions

No significant difference in heat resistance of *L. monocytogenes* was observed between slow (≤0.6°C $min^{-1}$) and rapid heating in sous vide cooked beef with pH of less than 5.8. However, in high pH beef (6.2) increase in heat resistance may be expected as a result of slowly rising temperature or heat shock.

## Literature


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