

## Effect of reduced nitrite content on the development of *Listeria monocytogenes* in pasteurized meat products.

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**Key Words:** cured meat products, reduced nitrite, shelf life, challenge test, *Listeria monocytogenes*.

### Introduction

In the framework of the project “Reduction of the use of nitrite in the preparation of organic meat products” a literature study has been performed followed by a challenge test with respect to the possibility of development of *Clostridium botulinum* in meat products with reduced nitrite content. Besides *Clostridium* also *Listeria monocytogenes* might pose a food safety risk in prepacked (sliced) cooked meat products distributed under refrigeration. Not clear is whether this risk might increase when the quantity of nitrite in the basic meat product is decreased. A challenge test has been performed with two industrially produced meat products, i.e. a cooked ham and a Bologna type sausage, with standard and reduced nitrite content.

### Materials and methods

Organic meat products, a cooked ham product and Bologna type sausage, were produced by two different Dutch meat processing companies. Beside the standard recipes, containing approximately 160 mg nitrite per kg product (ingoing content), comparable products were made with reduced nitrite content, i.e. 80 and 40 mg/kg product. The products were hygienically sliced and subsequently vacuum packed.

### Challenge test

Each meat product with different nitrite content was inoculated with a cocktail of three types of *Listeria monocytogenes*: type 1/2a (ATCC 35152), type 4a (ATCC 19114) and type 4b (ATCC13932). Before inoculation the cultures, kept on slants in refrigerator, were pre-cultivated twice in Brain Heart Infusion broth (BHI, Oxoid CM225) for 24 hours at 30°C. The full grown cultures were diluted in physiological peptone saline (PPS) to obtain a mixture at the desired level. A quantity of the meat product (ca. 1000 g) of each composition was placed in the bowl of a disinfected laboratory cutter and inoculated with 10 ml of a suspension of mentioned bacteria to a final level of about 10<sup>3</sup> per g product. After inoculation, the meat products were minced and homogenized for 2 minutes. Subsequently, the minced product was divided into 20 portions of 40 g and vacuum packaged in plastic pouches and stored at 7°C for up to 32 to 35 days.

### Microbiological analysis

At appropriate time intervals, samples of the minced meat product of each batch were taken in duplicate for microbiological analyses. From each single package a sample of 20 g was taken aseptically, diluted 10-fold in PPS and homogenized in a stomacher for 1 minute. Additional serial dilutions were made in PPS. Numbers of *L. monocytogenes* were determined using Palcam agar (Oxoid CM877 and SR150) as mentioned in ISO 11290-2: 1998. Plates were incubated at 37°C for 2 days. Aerobic colony counts were performed according to ISO 4833:1991 on Tryptone Soya Agar (TSA, Oxoid CM131) incubated at 30°C for 3 days. At the start of the challenge tests *a<sub>w</sub>* and pH values were measured of each type of product, at the end of the storage period the pH value was measured again.

## Results & Discussion

### Ham products

In all ham products with nitrite contents of 157, 80 or 40 mg/kg the inoculated *L. monocytogenes* bacteria increased by a factor 100 (2 log units) in circa 1, 5 weeks storage at 7°C (see Figure 1). Also the aerobic plate counts, mainly consisting of lactic acid bacteria, did increase rather fast in all ham products, exceeding a generally accepted maximum level of 10<sup>7</sup> (log 7) colony forming units (cfu) per g within 2 weeks 7°C (see Figure 2). Differences in growth rates for both bacteria between hams with standard, medium or low nitrite contents were not significant. The relative rapid growth of both *L. monocytogenes* and lactic acid bacteria can be attributed to the rather high, but quite common, water activity (*a<sub>w</sub>* value) of the ham products (*a<sub>w</sub>* = 0,972).

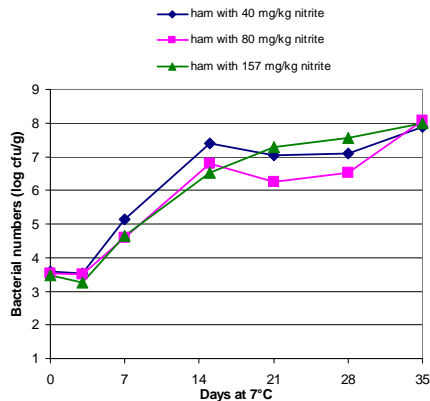


Figure 1 Development of *L. monocytogenes*

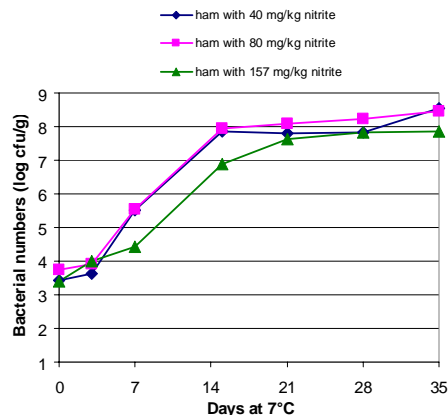


Figure 2 Development of aerobic bacteria

### Bologna type sausages

*L. monocytogenes* inoculated in Bologna type sausage with 160 or 80 mg/kg nitrite did not increase during the entire storage period of 32 days at 7°C (see Figure 3). In the Bologna type sausage with 40 mg/kg nitrite a rather small increase of *L. monocytogenes* by a factor 5 (0,7 log units) was seen during mentioned storage period. The aerobic plate counts in all sausages, mainly consisting of lactic acid bacteria, increased to a level of  $10^7$  cfu per g in 3 to 4 weeks (see Figure 4). The reason for the better microbiological stability of the Bologna type sausage compared to the ham product is the relative low water activity ( $a_w = 0.965$ ). Furthermore the presence of lactate in the products is known to have anti-listerial activity.

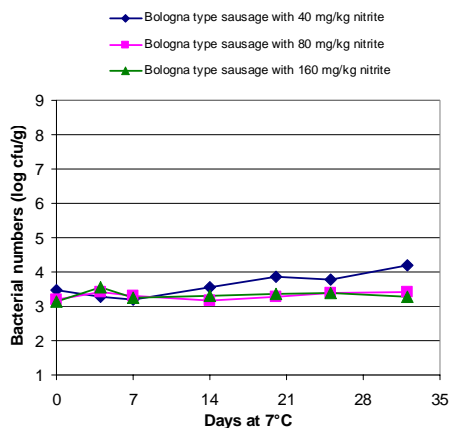


Figure 3 Development of *L. monocytogenes*

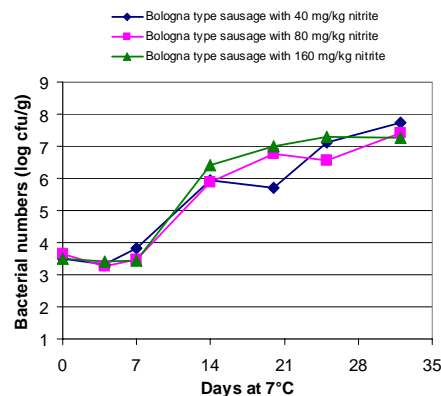


Figure 4 Development of aerobic bacteria

### Conclusions

- From the challenge test with cooked pork ham and Bologna type sausage with standard, medium and low nitrite levels it can be concluded that *L. monocytogenes* as well as lactic acid bacteria are not particularly sensitive to nitrite. Differences in growth rate of these bacteria in both products with different nitrite contents were small.
- Differences in water activity ( $a_w$  value) between the ham products and the Bologna type sausages appeared to have a more pronounced effect on inhibition of lactic acid bacteria, whereas the relative low water activity in combination with the presence of lactate in the Bologna type sausage almost completely inhibited the growth of *L. monocytogenes* during the storage period of 32 days at 7°C.
- For meat products with higher water activity and/or absence of lactate *L. monocytogenes* can only be controlled by prevention of contamination due to stringent hygiene during production.

### References

Stegeman, D., T.J. Verkleij, and F.K. Stekelburg (2006) *Reduction use nitrite producing organic meatproducts*. Report AFSG 658 - ISBN 9085850185.