A new method to enumerate low level of *Listeria monocyogenes* and *Salmonella* spp. in meat products

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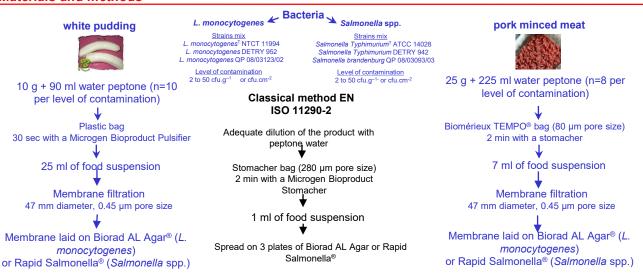
Introduction

The European Community Regulation No 2073/2005 defined a quantitative limit for 100 cfu.g^{-1} which was applicable for certain types of food during their shelf life. However an adequate counting method for low contaminating levels is still lacking. The EN ISO 11290-2 European and International Standard method for enumeration of *Listeria monocytogenes* is characterized by a limit of enumeration of 10-100 cfu.g^{-1}, and a poor accuracy. Recently a new method has been developed to enumerate *L. monocytogenes* at low contamination levels in several food matrices using a membrane filtration method with an enzyme/surfactant treatment to solubilize food components which increases handling period.

Objectives

The aim of the present work was to develop a new method to enumerate *L. monocytogenes* and *Salmonella* spp. at low concentration on meat products. This method is based on membrane filtration method without using treatment to solubilize food components.

Materials and methods

white pudding

10 g + 90 ml water peptone (n=10 per level of contamination)
↓
Plastic bag
30 sec with a Microgen Bioproduct Pulsifier
↓
25 ml of food suspension
↓
Membrane filtration
47 mm diameter, 0.45 μm pore size
↓
Membrane laid on Biorad AL Agar® (*L. monocytogenes*)
or Rapid Salmonella® (*Salmonella* spp.)

Bacteria

L. monocytogenes ← Bacteria → *Salmonella* spp.

Strains mix
*L. monocytogenes*T NTCT 11994
L. monocytogenes DETRY 952
L. monocytogenes QP 08/03123/02
Level of contamination
2 to 50 cfu.g^{-1} or cfu.cm^{-2}

Strains mix
Salmonella Typhimurium^T ATCC 14028
Salmonella Typhimurium DETRY 942
Salmonella brandenburg QP 08/03093/03
Level of contamination
2 to 50 cfu.g^{-1} or cfu.cm^{-2}

Classical method EN ISO 11290-2

Adequate dilution of the product with peptone water
↓
Stomacher bag (280 μm pore size)
2 min with a Microgen Bioproduct Stomacher
↓
1 ml of food suspension
↓
Spread on 3 plates of Biorad AL Agar or Rapid Salmonella®

pork minced meat

25 g + 225 ml water peptone (n=8 per level of contamination)
↓
Biomérieux TEMPO® bag (80 μm pore size)
2 min with a stomacher
↓
7 ml of food suspension
↓
Membrane filtration
47 mm diameter, 0.45 μm pore size
↓
Membrane laid on Biorad AL Agar® (*L. monocytogenes*)
or Rapid Salmonella® (*Salmonella* spp.)

Results

• For white puddings, the combination of the Pulsifier method and membrane filtration achieves detection limit close to 1 cfu.cm^{-2} for surface contamination

• For minced meat, the threshold is higher because the membrane is clogged quickly (after 7 ml of filtration) with the stomacher methods and Tempo bags

• The clogging of the membrane limit the maximum inoculation level of minced pork meat at 20 cfu.g-1

• The correlation between the inoculation level and the concentration after filtration is high for both products

• More experiences are needed to improve the detection limit close to 1 cfu.g^{-1} for minced pork meat

Enumeration of low level in white pudding
♦ *L. monocytogenes* $R^2 = 0.988$
■ *Salmonella* spp. $R^2 = 0,991$

Enumeration of low levels on minced pork meat
♦ *L. monocytogenes* $R^2 = 0.989$
■ *Salmonella* spp. $R^2 = 0,999$

Conclusions

This new method can be used to enumerate low level of bacteria on meat matrices. The ability to count low numbers is very useful for simulating the contamination level commonly found in contaminated products from food industries.