ISI FOOD PROTECTION

CENTRE OF EXPERTISE FOR
APPLIED FOOD MICROBIOLOGY

Laboratory report:

Challenge study in salami-type sausages - efficacy test with a protective culture to reduce risks caused by *Listeria monocytogenes*

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TARGET & TEST-DESIGN:

The use of protective cultures in salami-type sausages to minimize hygienic risks caused by *Listeria monocytogenes* is well established within the meat industries. For this purpose, there are several anti-listerial cultures commercially available. However, these protective cultures can be quite different in respect of their efficacy *in situ*, depending, for which recipes and fermentation conditions these cultures are applied.

Aim of this study was, to document the anti-listerial efficacy of a protective culture in a standard recipe under industrial production conditions according the EU guidelines for conducting challenge tests in ready-to-eat (RTE) foods.

Cultures and Listeria pool:

For this challenge tests, following cultures were applied:

- Protective culture: **M-CULTURE Safe 3100 SSL** (internal sample number: NG-578)
- Starter culture **M-CULTURE SA 28-100** (internal sample number: NG-580)

For contaminating the meat batter, a standardized and cold-adapted pool of containing approximately equal numbers of each of the following four Listeria strains was used:

- *Listeria monocytogenes* (ISI 20, 21, 22); isolated from meat products
- *L. monocytogenes* reference strain from the ATCC culture collection, clinical isolate (ATCC 7644, ISI 26)

Production of the sausages as well as fermentation was conducted under industrial conditions in a L2-classified pilot plant. The fermentation parameter, the recipe and the dosage of protective and starter culture were specified by the customer.

Preparation of the sausages:

Frozen, standardized meat (Pork shoulder, 21% in final recipe; and pork back fat, 23% in final recipe), spice mix and cultures were delivered by the customer. Frozen meat and fat together with the spice mix and the cultures were chopped (to 3 mm meat particle size). That followed, fresh meat (pork shoulder, 3 mm; 56%) was mixed in, and at the end of the cutter-process, salt was added.

The meat batter was filled into casings (45 mm) and the sausages were fermented by a ripening program that is standard for German type salami-sausages.

Microbiological analyses:

The listeria counts were determined at day 0 (t_0 = recovery rate in the final meat batter), after 24 h (critical fermentation period = lag phase) and after 5 days. All analyses were carried out in triplicates. Salami samples without protective cultures were used as reference samples.

- Qualitative detection of *Listeria monocytogenes in situ*

ISO 11290-2:1998 Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of *Listeria monocytogenes* - Part 2: Enumeration method with amendment ISO 11290-2:1998/Amd 1:2004 Modification on the enumeration media. ALOA agar. Duplicate plating per sample as specified in ISI 7218:2007 Microbiology of food and animal feeding stuffs - General requirements and guidance for microbiological examinations.
Detection limit: 5 CFU/g

In addition at day 5:

- ☐ Qualitative determination of *Listeria monocytogenes in situ in 25 g* according ISO 11290-1
  Detection limit: 1 CFU/25g

Complimenatry analyses:

- ☐ pH (core) for controlling the fermentation process
- ☐ Water-activities (a_w-values)
- ☐ Weight loss

■ Sampling points and number of samples*

	Day 0 Recovery rate	24 h	5 days
Salami with protective culture	2	3	3
Salami without protective culture	2	3	3
In total:	**4**	**6**	**6**

**Determination of listeria counts in duplicates*

■ related documents

[1] Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. ANNEX 1, Chapter 1: Food safety criteria.
[2] SANCO/1628/2008 ver. 9.3 (26112008) Guidance Document on *Listeria monocytogenes* shelf-life studies for ready-to-eat foods, under Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs
[3] Technical Guidance Document on shelf-life studies for *Listeria monocytogenes* in ready-to-eat foods. CRL for *Listeria monocytogenes* (Community Reference Laboratory), 14/11/2008.

TEST RESULTS:

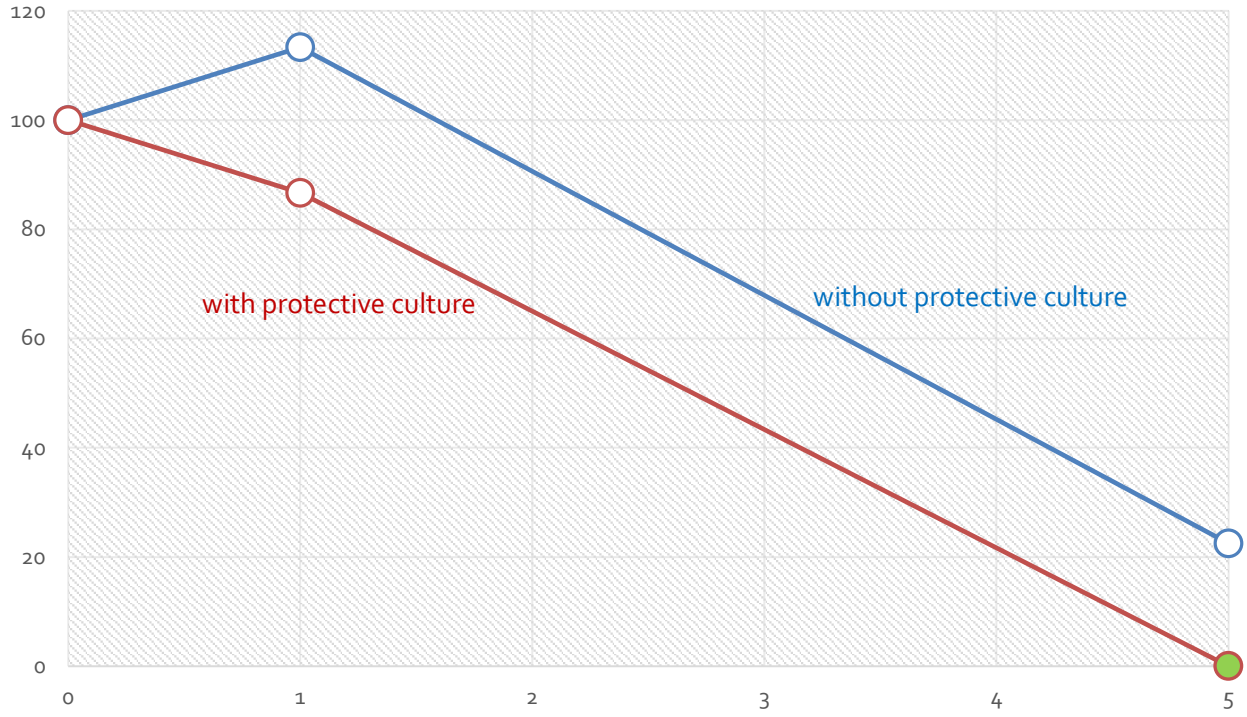

Fig.: Listeria counts [CFU/g] during fermentation within the first five days.

● After five days fermentation, no listeria were detectable in 25 g (according ISO11290-1) in all samples that were treated with the protective culture.

Comments on the results:

The application of the protective cultures had two main effects with a positive **impact on food safety**:

1: The listeria counts were already reduced within the lag phase (first 24 h of fermentation), were food safety is critical due to high pH and a_w-value. In the non-protected samples, a slight increase of the listeria counts was detectable.

2: Apart from that and of greater relevance regarding food safety is the fact, that already after 5 days fermentation, all samples with the added protective culture were negative in 25g, whereas in the non-protected samples, *Listeria monocytogenes* was still detectable on levels of about 20 cfu/g (see Fig. above, listeria counts at day 5).

ISI fast facts:

- Highly specialised on applied food & plant microbiology
- L3* classified food safety laboratories & L3* classified food pilot plant
- Cross-industrial & along the food value chain
- International customer (food processors) portfolio
- Accredited according ISO 17025
- Comprehensive strain collection of food spoilage microorganisms as well as of food pathogens (e.g. *Salmonella*, *E. coli* O157, *Campylobacter*, *Listeria monocytogenes*)
- Approval for working with *Clostridium botulinum*

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