CULTURES TASTE TECHNOLOGY
MADE IN GERMANY

Meat Cracks®
Components for good meat
Technologie GmbH

PROTECTIVE CULTURES

for raw sausages, spreadable raw sausages, raw cured bellies and Bacon

The protective culture M-CULTURE® Safe 3100 SSL in combination with the activation medium Meat Safe MC-RE-200 ▶▶

SAFE
ListeriaProtection

The protective culture
M-CULTURE® SAFE 3100 SSL

Protection cultures are especially designed products which are adapted for protection against pathogenic microorganisms (e.g. Listeria).

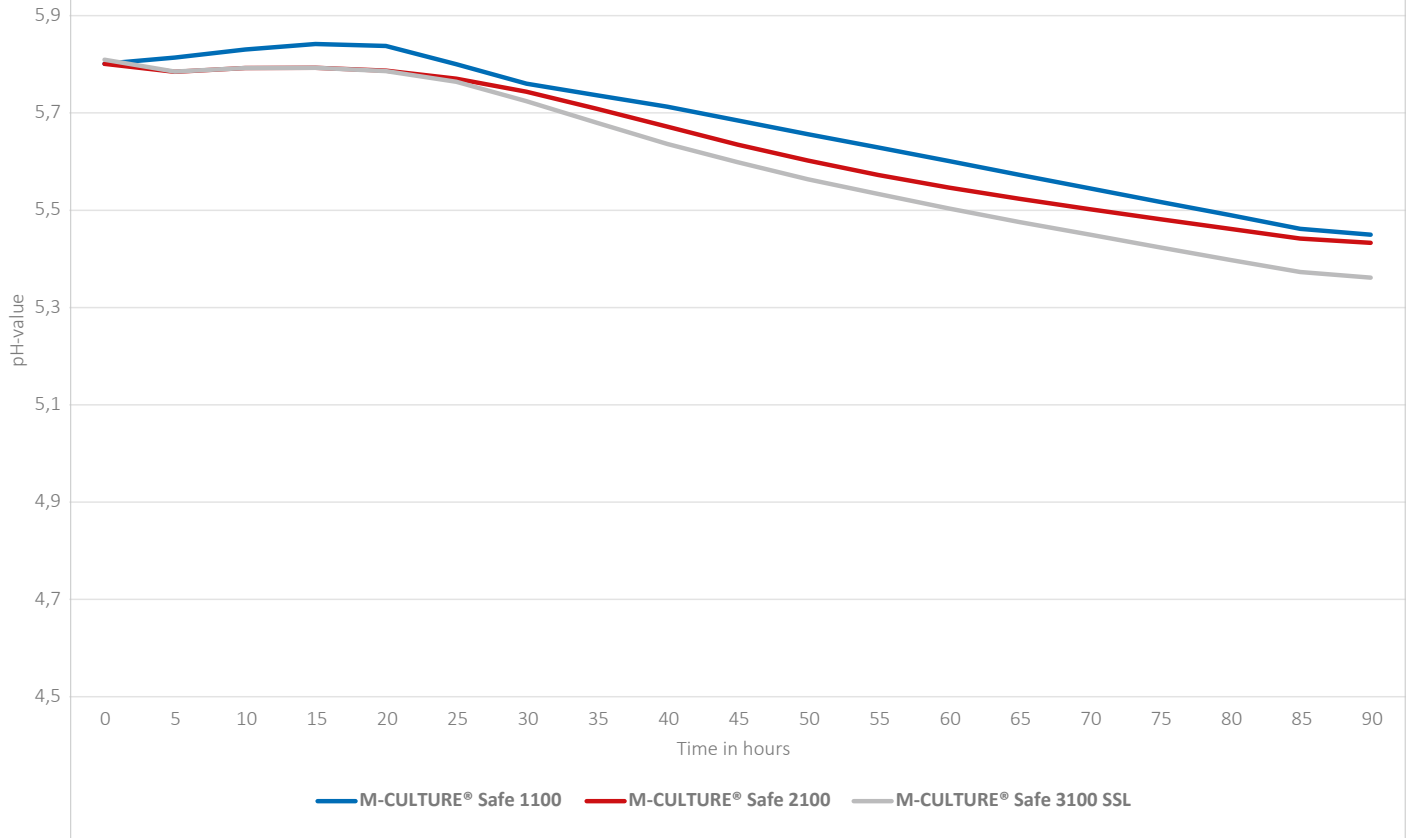
According to law, legislation requires that the Listeria cell count does not exceed the limit value of 100 cfu/g during the whole shelf life of the product. Products which promote listerial growth are subject to stricter regulations. If no satisfying proof exists for the competent authority that the product does not exceed the limit value of 100 cfu/g during its whole shelf life, the zero tolerance limit is valid. If there is proof to the satisfaction of the competent authority, e.g. an existing challenge test, the limit value of 100 cfu/g is valid.

Meat Cracks Technologie GmbH offers its customers especially designed M-CULTURE® protection cultures which guarantee maximum product safety. Our experienced Meat Cracks technologists will be glad to provide further information.

M-CULTURE® SAFE [SAFETY]
PROTECTIVE CULTURES

Art.-No.	Name	Composition	Application	Packaging
44.00154 44.00157	**M-CULTURE® Safe 1100**	• K varians • Staph carnosus • Lb curvatus • Staph xylosus	– For all types of sliceable and spreadable raw sausages requiring safe protection against listeria and mild acidification – Protective strain: Lb curvatus	25 g (for 100 kg) 50 g (for 200 kg)
44.00254	**M-CULTURE® Safe 2100**	• Staph xylosus • Staph carnosus • Lc lactis • Lb plantarum	– For all types of sliceable and spreadable raw sausages requiring safe protection against listeria and mild acidification – Protective strain: Lb plantarum	20 g (for 100 kg)
44.00374 44.00379 44.00380 44.00381	**M-CULTURE® Safe 3100 SSL**	• Staph xylosus • Staph carnosus • Lc lactis • Lb plantarum • Lb curvatus • K varians	– For all types of sliceable (in combination with **Meat Safe MC-RE-200** also for all types of spreadable) raw sausages, requiring safe protection against listeria and mild acidification – Protective strains: Lb plantarum, Lb curvatus **– Top-Seller** – Very good bacteriocin formation ability	20 g (for 100 kg) 40 g (for 200 kg) 100 g (for 500 kg) 200 g (for 1000 kg)

Acidification curves of some Meat Cracks rotective cultures:



The activation medium
MEATSAFE MC-RE-200

Simple and secure handling

The application was developed in such a way that the fermentation can be carried out at room temperature (22°C). For this purpose dissolve the medium MeatSafe MC-RE-200 in water and add the protection culture M-CULTURE® Safe 3100 SSL. After a fermentation time of 24 to 26 hours the culture can be added directly to the product.

Simple quality control

By measuring the final pH-value of the culture after fermentation it can be guaranteed that the fermentation was successful. The pH-value should be between pH 4,0 and 4,2 after 24-26 hours.

Optimized bacteriocin production

During fermentation, the cultures of the protection culture
M-CULTURE® Safe 3100 SSL are pre-activated to get the optimal use of their anti-listerial effect in the product (see figure on the right).

Optimum activity spectrum of the produced bacteriocines

The produced bacteriocines are able to inactivate all types of Listeria strains, without damaging the useful flora (e.g. lactic acid bacteria) (see figure next page).

No duty of declaration

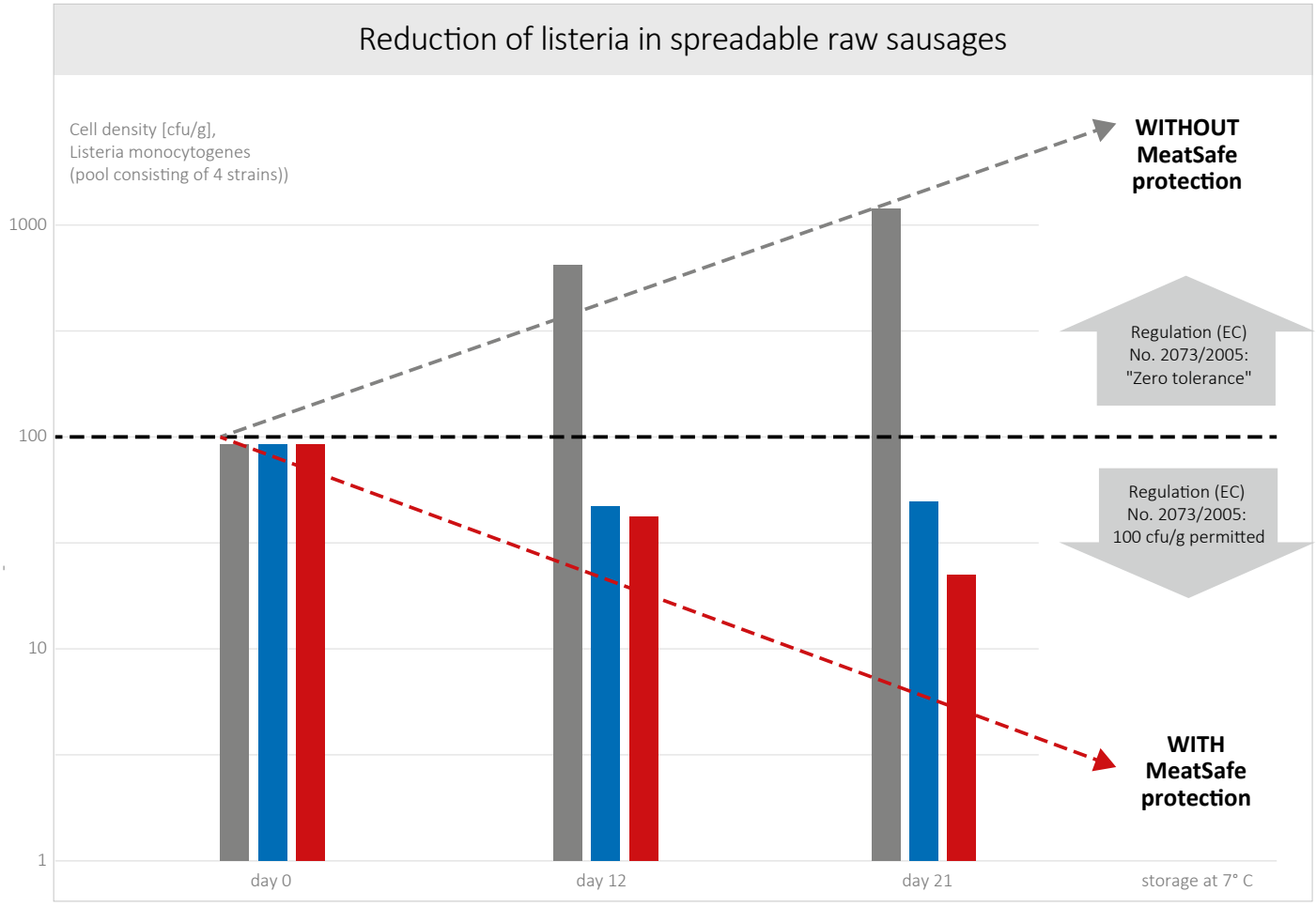
The product MeatSafe MC-RE-200 is a technical aid which has no functional properties in the product and its ingredients disappear during incubation time.

MEATSAFE MC-RE-200 [ACTIVATION MEDIUM]

According to the EU-regulation no. 2073/2005 on microbiological criteria for foodstuffs, legislation requires that the listeria cell count does not exceed the limit value of 100 cfu/g during the whole shelf life of the product. Therefore, improved listeria protection is required for many meat products.
Against this backdrop, Meat Cracks has developed the activation medium MeatSafe MC-RE-200 for the microbiological stabilization of spread-
able raw sausages and raw cured belly. It is specially tailored to be used in conjunction with the protective culture M-CULTURE® 3100 SSL.

The limited bacteriocin formation ability of M-CULTURE® 3100 SSL at lower temperatures made it necessary to develop a medium guaranteeing safe protection against listeria in products which are fermented at low temperatures.
The specially designed activation medium MeatSafe MC-RE-200 is characterized by simple and secure handling, simple quality assurance, optimized bacteriocin formation, optimal efficacy spectrum and no duty of declaration.

The following figure reveals that a reduction of the listeria cell density during cold storage could be achieved through the addition of the activation medium. By contrast, the reference sample exhibited an increase in the listeria cell density.
With regard to the legal regulation on the permitted limit values of listeria, the good bacteriocin formation ability of the Meat Cracks protective culture allows for a broad range of possible applications in the area of ready-to-eat products. Here growth of listeria is possible due to the pH- values and a_w-values.
For a specific product, we always recommend to carry out a challenge test, in order to document the listeria killing effect according to the EU-regulation no. 2073/2005.



THE EU-REGULATION NR. 2073/2005

regarding Listeria monocytogenes

CATEGORY 1.1
Ready-to-eat food
– for infants
– for special medical purposes

CATEGORY 1.2
Ready-to-eat food which can promote the multiplication of Listeria monocytogenes
CATEGORY 1.3

Ready-to-eat food which cannot promote the multiplication of Listeria monocytogenes. These include food with a:
– pH-value < 4.4 or a_w-value < 0.92
– pH-value < 5.0 and a_w-value < 0.94
– Shelf life < 5 days

Before leaving the immediate control of the producer

MISSING satisfactory evidence for the competent authority in compliance with the limit value of 100 cfu/g during the whole shelf life.

EXISTING satisfactory evidence for the competent authority in compliance with the limit value of 100 cfu/g during the whole shelf life.

ZERO TOLERANCE
Listeria monocytogenes must not be detected in 25g

LIMIT VALUE 100 cfu/g

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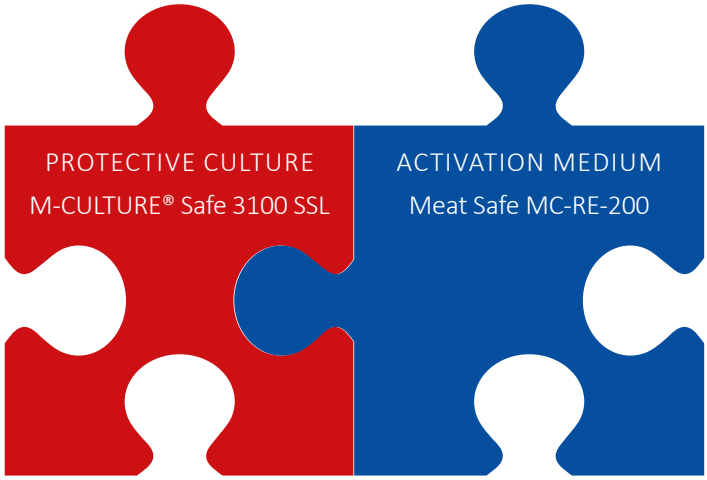


ISI FOOD PROTECTION

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*

21.06.2017

Prof. Dr. Dieter Elsser-Gravesen

ISI FOOD PROTECTION ApS
Agro Food Park 13
DK-8200 Aarhus N
cvr 3266664

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:

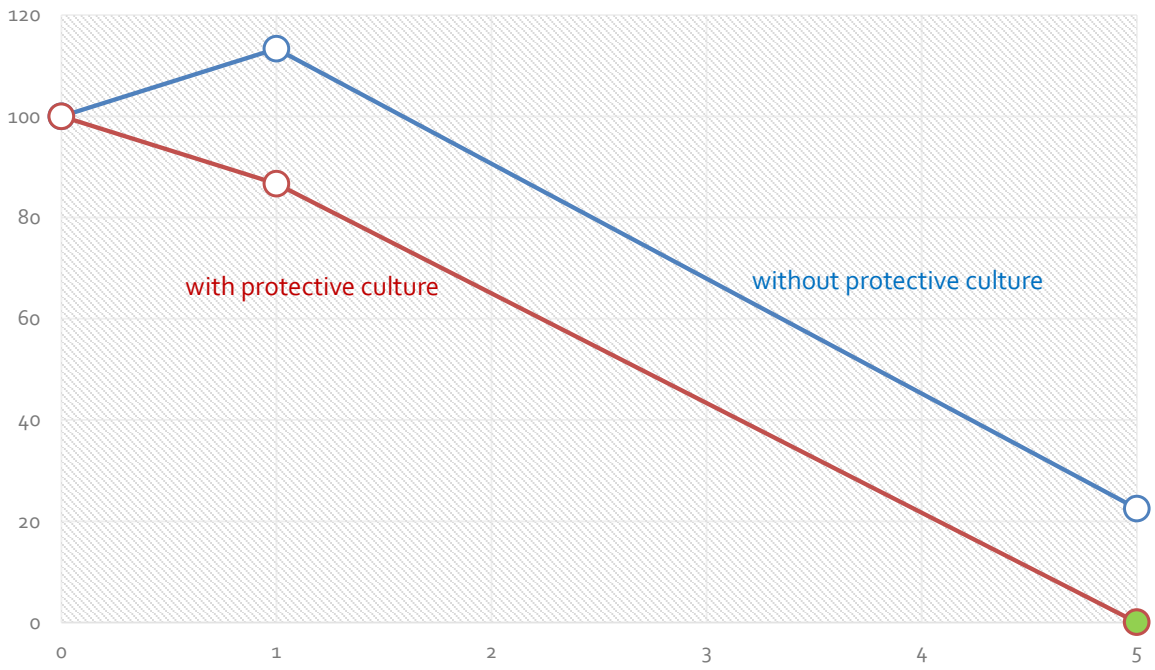


Fig.: Listeria counts [CFU/g] during fermentation within the first five days.

● 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**:

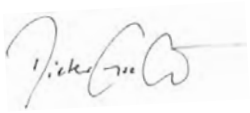
1: The listeria counts were already reduced within the lag phase (first 24 h of fermentation), were food safety is critical due to high pH and a_w-value. In the non-protected samples, a slight increase of the listeria counts was detectable.

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*

Aarhus, 21 June 2017



Dr. Dieter Elsser-Gravesen

MANAGING DIRECTOR ISI FOOD PROTECTION
AFFILIATE ASSOCIATE PROFESSOR IN FOOD MICROBIOLOGY AARHUS UNIVERSITY

ISI FOOD PROTECTION

CENTRE OF EXPERTISE FOR
APPLIED FOOD MICROBIOLOGY

Laboratory report:

Production of an activation culture for Challenge studies ZMW

27.11.2018

Prof. Dr. Dieter Elsser-Gravesen

ISI FOOD PROTECTION ApS
Agro Food Park 13
DK-8200 Aarhus N
cvr 3266664

Basics:

For carrying out the challenge studies with Listeria and Salmonella in Zwiebelmettwurst, the protective culture *M-Culture Safe 3100 SSL* was pre-cultivated in the medium MeatSafe MC-RE-200 according to the instructions of the Meat Cracks Technologie GmbH to directly incorporate a sufficient amount of bacteriocins into the meat.

The aim of the pre-tests was to determine at which point during fermentation the highest yields and antilisterial activities can be achieved.

In the following, after internal agreement with Meat Cracks, the ZMW-meat should be treated with three different dosages:

Test batch 1:	10	ml/kg	1.0 %
Test batch 2:	15	ml/kg	1.5 %
Test batch 3:	20	ml/kg	2.0 %

ISI Results:

1. Determination of bacteriocin yields as a function of fermentation time

The cultures were added to the growth medium according to the product specification and fermented at 22°C for 25 hours.

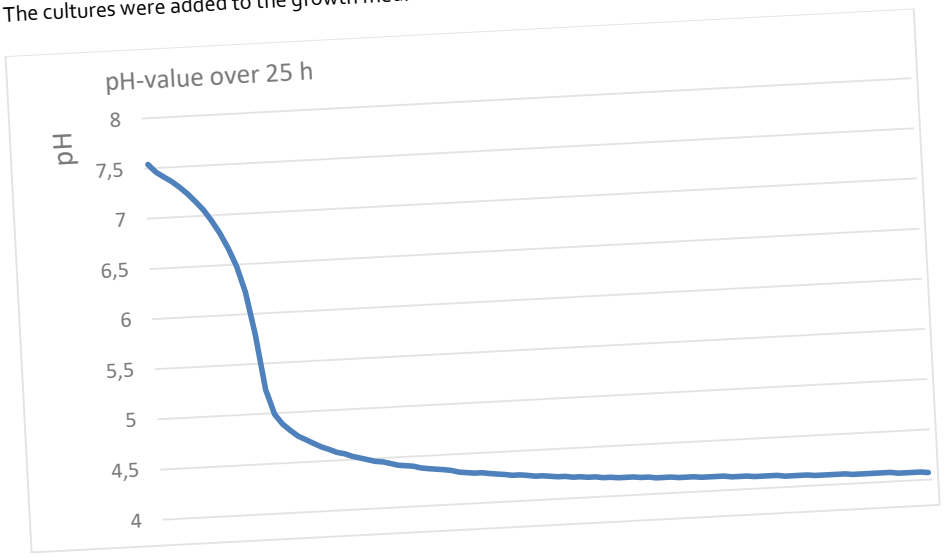


Fig. 1: pH-value over a total period of 25 h

During fermentation, a part of the fermentation broth was analyzed after 22h, 23h, 24h and 25h and the bacteriocin activity was evaluated in a well-diffusion-assay and the cell densities were also determined.

- ☐ Determination of bacteriocin activity in accordance with ISI 71660
- ☐ Determination of cell densities of lactic acid bacteria in accordance with ISI 71210

	Bacteriocin activity	Cell densities LAB
Fermentation time	Inhibition zone [mm]	[cfu/g]
22h	21.87	1.30E+08
23h	23.33	1.23E+09
24h	21.59	1.27E+09
25h	20.11	1.43E+09

Table 1: Yields of cell densities and antilisterial activity after different fermentation times

↳ By means of these fermentation tests, it was defined that the optimal fermentation time (highest bacteriocin activity) is **23h at room temperature.**

2. Addition of different concentrations of the fermentation broth to the ZMW meat

For the challenge studies it was decided to use different dosages of the 23h fermentation broth. For this purpose, fermentation was carried out on a large scale (1000ml) and the bacteriocin activities were evaluated. Here, the same bacteriocin activities were determined as in the pre-tests.

During the challenge study the impact of the dosage on the listeria was identified as follows:
Contamination densities (day 0): 77 cfu/g (LOG 10 cfu/g:1.89)

Day 2 (after maturation):

	Determination a [cfu/g]	Determination b [cfu/g]	Total [cfu/g]	Reproduction potential δ
Batch 1: **1.0 %**	90	100	95	0,09
Batch 2: **1.5 %**	40	50	45	Negative
Batch 3: **2.0 %**	20	20	20	Negative

↳ A clear dependence of the dosage can be identified after a short „maturation phase" of the Zwiebelmettwurst. With an addition of 1.0% of the fermentation broth to the meat a slight increase of listeria is detected. An addition of 1.5% led to a reduction. This reduction is significantly higher with an addition of 2.0%.

Day 4

	Determination a [cfu/g]	Determination b [cfu/g]	Total [cfu/g]	Reproduction potential δ
Batch 1: **1.0 %**	30	10	20	Negative
Batch 2: **1.5 %**	30	30	30	Negative
Batch 3: **2.0 %**	20	20	20	Negative

↳ On day 4, no differences are noticeable; the listeria densities of all dosages are at the same low level.

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MADE IN GERMANY

Meat® Components for good meat Cracks Technologie GmbH

Meat Cracks Technologie GmbH

Rienshof 2
D-49439 Steinfeld-Mühlen
Germany

+49 5492 9609-0

+49 5492 9609-29

info@meatcracks.de

www.meatcracks.de

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