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RF-cooking SUSFOOD

Microbial inactivation during heat treatment with traditional cooking and RF-cooking

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Summary

Back-ground

The overall aim of this project is to develop a new RF-cooking equipment with a more uniform heat distribution. The equipment has been developed at Fraunhofer.

The aim of this work is to demonstrate how efficient the microbial inactivation is in RF-cooked ham and to compare the inactivation with traditionally cooked ham heated to 65°C/30 minutes and 72°C/2 minutes.

Conclusion

Traditional heating to 72°C for 2 minutes (F_{70}=105 minutes) efficiently inactivated the cocktail of bacteria added.
Traditional heating to 65°C for 30 minutes (F_{70}=13 minutes) did not inactivate all bacteria in the cocktail added.
RF-cooking with 360 kJ and 380 kJ combined with **heating in water bath** at 74°C to 72°C did not inactivate all bacteria in the cocktail added.

After 2 months of storage at 5°C, survivors from the 65°C heat treatment increased to 5-7 log cfu/g. 16S sequencing indicated that the growth was caused by *E. faecium.* In ham heated to 72°C, no growth or a slight increase in bacteria was observed. The number was too low for 16S sequencing. In RF-cooked hams, only one cold spot was detected. The survivors increasing to 4 log cfu/g were dominated by *Lb. sakei.*

After 2 months of storage at 8°C, survivors from the 65°C heat treatment increased to 6-8 log cfu/g. 16S sequencing indicated that the growth was caused by *Lb. sakei* and *E. faecium.* In ham heated to 72°C, a slight increase in bacteria was observed. Hams with approx. 3 log cfu/g were analysed by 16S sequencing. This showed that *Lb. sakei* dominated the microbiota. In RF-cooked hams, one ham cooked at 380 kJ showed 3 log cfu/g in the gel. The sequencing indicated that these organisms were dominated by *Lb. sakei*.

The cooking loss was higher in hams RF-cooked at 380 kJ compared to 360 kJ. The cooking loss was higher in hams cooked to 72°C compared to hams cooked to 65°C. The order of cooking loss was:

65°C (5.2%) < 72°C (7.7%) ≈ 360 kJ (8.4%) < 380 kJ (14.5%)

Introduction

Background

The overall aim of this project is to develop a new RF-cooking equipment with a more uniform heat distribution. The equipment has been developed at Fraunhofer.

The aim of this work is to demonstrate how efficient the microbial inactivation is in RF-cooked ham and to compare the inactivation with traditionally cooked ham heated to 65°C/30 minutes and 72°C/2 minutes.

Materials and methods

Strains used

The literature shows huge variation in D-values among bacteria. For *E. coli* O157:H7 the D_{57}-value varies between 2 and 40 minutes. For Salmonella, D_{58}-values vary between 2 and 22 minutes, and for *L. monocytogenes,* D_{58}-values vary between 6 and 50 minutes.

In the literature survey (Koch, 2016), it was found that enterococci are among the most heat resistant vegetative cells relevant to processed meat heated to 72-75°C. At this temperature, inactivation of spores from *Clostridium* spp. and *Bacillus* spp. is not relevant to discuss as these spores are not inactivated until a temperature of 90°C or more is reached.

For this work, different *Enterococcus* strains were chosen as they represent some of the most heat resistant strains relevant to processed meat. Strains from the DMRI Culture Collection were chosen because of the heat resistance shown in a pre-experiment ensuring that the strains used represent different heat resistance and cover the heat resistance among pathogenic bacteria like *Listeria monocytogenes* and *Salmonella*.

If the challenge test shows that the RF-cooking is capable of inactivating the enterococci used in this cocktail then the process is safe for industrial use.

The following strains were used for the experiment.

- *Enterococcus faecium* DMRICC 4266, D_{65} = 55 min.; D_{68} = 17 min.
- *Enterococcus faecalis* DMRICC 4168, D_{60} = approx. 8 min, D_{65} = approx. 0.6 min.
- *Enterococcus durans* DMRICC 4371[a)], D_{65} = 17 min
- *Brochotrix termospachta* DMRICC 4738, D_{55} = 0.86 min.
- *Lactobacillus sakei* DMRICC 3852, D_{60} = 0.33 min
- *Strep. thermophilus* DMRICC 5010, D_{65} = 11.8 min., D_{70} = 0.8 min.

[a)] 16S sequencing indicates that this culture contains two strains: *E. faecium* and *Lb. sakei* (Table 20).

Production of ham

Pork leg was deboned at DMRI and used for curing. The cured hams were produced with 15% weight gain reaching 2% salt and 60 ppm nitrite.

Trimmed pork leg muscles were cut into small pieces using a double kidney plate (knife). Then cured overnight by tumbling in vacuum at 5°C for 6 hours with 5 minutes of rotation (6 rounds per minute), 5 minutes of rest. Rest at 5°C until the next morning. Tumbling for 15 minutes before filling into casings. At the start of curing, the bacteria cocktail was added.

Casings from Fraunhofer (PA-material, 70 µm thickness) were filled with the inoculated cured meat. Diameter: 100 mm, length: 35 cm, weight: 2.8-2.9 kg.

Heat treatment

The ham was divided into three groups:

A) Traditional cooking at DMRI (72°C/2 minutes)
B) Traditional cooking at DMRI (65°C/30 minutes)
C) RF-cooking at Fraunhofer (for details see Appendix 1)

Cooking yield

The cooking loss was measured on hams used for chemical and microbial analysis.

Cooking loss = (total weight – casing – ham) * 100 /(total weight – casing)

Chemical analysis

300 g of each sample was used to analyse pH, salt and water.
The methods used was:

pH: Mod.a. ISO 2917 pH in meat and meat products: Measurements of pH (reference method) and AOAC Official Method 981.12 pH of Acidified Foods. 1982. (DMRI SM 011)

NaCl: NMKL method no. 178, 2004: Chloride (salt). Determination in foods by potentiometric titration. (DMRI SM 018)

Water: Moisture and ash. Determination in meat and meat products. NMKL method no 23, 1974, 2nd Ed. (DMRI SM 002)

Microbial sampling

Microbial sampling was made for the following steps in the process:

- Each culture used for the inoculum
- The inoculum cocktail
- Ham before curing and inoculation
- Inoculated cured ham before stuffing into the casing
- Inoculated cured ham in casing before heating
- Ham heated to 72°C
- Ham heated to 65°C
- Ham heated by RF-cooking
- Ham stored for 2 months at 5°C after the three different heat treatments
- Ham stored for 2 months at 8°C after the three different heat treatments

Microbial substrate and incubation

The following substrates and temperatures were used for the microbial analysis.

- Slanetz agar, (45°C, 48 hours) (P05018A, ThermoFischer/Oxoid)
- STA-agar (20°C, 5 days) (STAA-agar base CM0881 + STA-supplement SR0162, Oxoid)
- MRS-agar + 0.2 K-sorbat (20°C, 5 days) (CM1153, Oxoid)
- BHI-A (20°C, 5 days) (Brain Heart Infusion agar, CM1136, Oxoid)
- BHI-A (37°C, 24 hours) (Brain Heart Infusion agar, CM1136, Oxoid)
- BHI-A (45°C, 48 hours) (Brain Heart Infusion agar, CM1136, Oxoid)

Results

Transport, production, quality and yield

Temperature during transport

The temperature during transport to Fraunhofer and back to DMRI was measured by two loggers (one in each box):
Logger 1: mean temperature: 2.1 ± 1.2°C, max. temperature was 8-14°C during 30 minutes (26 April at 13.54-14.24).
Logger 2 (lost and broken package): mean temperature: 2.2 ± 1.7°C, max. temperature was 10°C during 10 minutes (3 May at 03.53-04.03).

The temperature abuse for hams in package two (logger 2) was:
3 May: 5-10°C for 1 hour
5 May: 8°C for 8½ hours

Chemical analyses

From each batch produced, 3 samples were analysed for pH, salt and water. The results (Table 1) show that the two batches of ham were very similar in preservatives (salt-in-water) and pH, which is important for comparing heat inactivation and growth of survivors in the two different batches.

Table 1. Chemical analysis of cooked ham. Batch 1: traditional cooking to 65°C and 72°C. Batch 2: RF-cooking.

Batch	pH	Salt %	Water %	Salt/water %
65°C	6.0 ± 0.0	2.1 ± 0.0	74.9 ± 0.4	2.8
72°C	6.0 ± 0.0	2.1 ± 0.0	75.2 ± 0.2	2.8
RF-cooking	5.9 ± 0.0	2.0 ± 0.0	74.1 ± 0.5	2.8

Cooking loss after heat treatment

The cooking loss (meat exudate/gel) was measured on 3-4 hams 5-7 days after heat treatment (Table 2+3).

Hams cooked to 72°C (F_{70}=105) had a cooking loss of 8.4 ± 1.3%. The texture of the cooking loss was like a thick gel.

Hams cooked to 65°C (F_{70}=13) had a cooking loss of 5.3 ± 0.2%. The texture of the cooking loss was like a gel, softer than the hams cooked to 72°C.

The RF-cooked ham (360 kJ or 380 kJ) had a cooking loss of 11.2 ± 4.4%. The texture of the cooking loss was watery. One of the untreated hams transported to Fraunhofer and back to DMRI was heated to 65°C in a water bath in the laboratory. This ham was cured/transported in a total of 18 days before heating and had a cooking loss of 13.2%.

It must be noted that the RF-cooked hams were produced from another batch of raw meat, and that the process time before heat treatment differed slightly compared to hams cooked to 72°C and 65°C. The hams cooked to 72°C and 65°C were cured for 4 days whereas the hams for RF-cooking were cured for 7 days before heating, and the extra hams cooked to 65°C were cured/transported/stored for 18 days.

During RF-cooking, two different levels of energy were used. Some hams were cooked at 360 kJ and others at 380 KJ. Both treatments for about 12 minutes. After that, all hams were surface treated in a 74°C hot water bath. The cooking loss was 6% higher during RF-cooking at 380 kJ compared to cooking at 360 kJ (Table 3).

Table 2. Cooking loss (%) after heating and after storage for 2 months.

	65°C	72°C	RF-cooking[b)]
After heating (n=4)	5.3 ± 0.2	8.4 ± 1.3	11.2 ± 4.4
After storage at 5°C/2 months (n=3)	4.8 ± 0.5	7.3 ± 0.9[a)]	9.5 ± 1.8
After storage at 8°C/2 months (n=3)	5.5 ± 0.8	7.4 ± 0.3	13.7 ± 4.7

a) One ham with a cooking loss of 16.9% is not included in the mean.
b) Ham cooked at 360 kJ and 380 kJ. See Table 3. The texture was loose/crumbly.

Table 3. Cooking loss (%) in RF-cooked hams at 360 kJ and 380 kJ.

	360 kJ	380 kJ
After heating	7.9; 6.9	14.3; 15.7
After storage at 5°C/2 months	7.5; 11.1	10.0
After storage at 8°C/2 months	8.4	17.4; 15.3
Mean ± std.	8.4 ± 1.6	14.5 ± 2.7

Visual appearance

The visual appearance of the hams differed (Figure 1).

The hams cooked to 72°C and 65°C had a uniform and firm texture, no holes were observed. The colour was bright/pale. They were made from meat batch 1 (Figure 1).

The RF-cooked hams had an un-uniform and soft texture, holes were observed. The colour was redder than for the hams cooked to 72°C or 65°C. They were made from meat batch 2 (Figure 1).

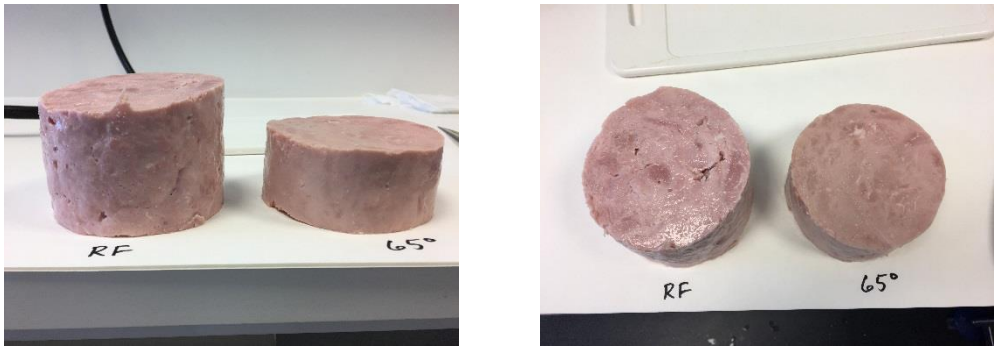


Figure 1. Ham cooked to 65°C (meat batch 1) and RF-cooked (meat batch 2)

Cooking loss after 2 months of storage at 5°C and 8°C

The cooking loss (exudate) was measured on 3-4 hams 2 months after heat treatment.

Hams cooked to 72°C (F_{70}=105) had a cooking loss of 7.3 ± 0.9% after storage at 5°C and 7.4 ± 0.3% after storage at 8°C. The texture of the cooking loss/exudate was like a thick gel.

Hams cooked to 65°C (F_{70}=13) had a cooking loss of 4.8 ± 0.5% after storage at 5°C and 5.5 ± 0.8% after storage at 8°C. The texture of the cooking loss/exudate was like a gel, softer than for the hams cooked to 72°C.

The hams RF-cooked at 360 kJ had a cooking loss of 7.5/11.1% after storage at 5°C, and 8.4% if stored at 8°C. RF-cooking at 380 kJ results in a cooking loss of 10% when stored at 5°C and 15.3/17.4% when stored at 8°C. The texture of the cooking loss/exudate was like water.

It must be noted that the RF-cooked hams were produced from another batch of raw meat, and that the process time before heat treatment differed slightly compared to hams cooked to 72°C and 65°C. The hams cooked to 72°C and 65°C were cured for 4 days whereas the hams for RF-cooking were cured for 7 days before heating.

Inoculation of ham

Microbial count in cultures

The number of bacteria in the cultures used varied between 8 and 9 log cfu/ml. The measured numbers are shown in Table 4 and 5.

Table 4. Number of bacteria (log cfu/ml) in the inoculum used for the cocktail (after centrifugation and resuspension in 0.9% salt water)

Bacteria	DMRICC	Heat 72 & 65°C BHI-agar/ selective agar	RF-cooking BHI-agar/ selective agar
Lb. sakei	3852	9.9 / 9.7	9.9 / 9.9
Ent. faecalis	4168	9.1 / -	9.5 / 7.7
Ent. faecium	4266	9.4 / -	9.5 / 9.4
Ent. durans	4371	9.4 / -	9.8 / -
B. thermospachta	4738	9.0 / -	9.5 / 9.3
Strep. thermophilus	5010	< 3	7.7 / -
E. coli	4135	9.5 / -	Not used
E. coli	4235	9.6 / -	Not used

-) not analysed

Table 5. Number of bacteria (log cfu/ml) in the cocktail used

Substrate	72 & 65°C	RF-cooking
BHI, 20°C/5 days	9.6	9.7
BHI, 37°C/1 day	9.4	9.5
BHI, 45°C/2 days	9.2	8.9
Slanetz, 45°C/2 days	8.8	8.8
STA, 20°C/5 days	7.8	8.7
MRS-S, 20°C/5 days	9.3	9.2

Theoretical inoculum of hams

The meat was inoculated during curing by adding 120 ml of the bacteria cocktail to 120 kg of meat+brine.

The theoretical inoculum per gram of cured ham is then: $4*10^9$ cfu/ml * 120 ml/120 kg = $4*10^9$ cfu/kg = $4*10^6$ cfu/g.

In Table 6, the theoretical inoculum of the different bacteria is shown. This shows that the different bacterial species added are at the same level ranging from 6 to 6.7 log cfu/g. The major difference is that to the cocktail used for the heating to 72°C and 65°C, *E. coli* was added but no *Streptococcus thermophilus*. And to the cocktail used for RF-cooking, *Streptococcus thermophilus* was added but no *E. coli.*

Table 6. Theoretical inoculum of the different species used in the experiments.

Bacteria	DMRICC	Heat 65 & 72°C BHI-agar / selective agar	RF-cooking BHI-agar / selective agar
Lb. sakei	3852	6.7	6.9
Ent. faecalis	4168	6.1	6.5
Ent. faecium	4266	6.4	6.5
Ent. durans	4371	6.4	6.8
B. thermospachta	4738	6.0	6.5
Strp. thermophilus	5010	Not used	4.7
E. coli	4135	6.5	Not used
E. coli	4235	6.6	Not used
Cocktail	all	6.6	6.7

Inoculum batch 1

The fresh meat used for production of hams for heating to 72°C and 65°C had an initial count of 4.3 ± 0.2 log cfu/g (BHI, 20°C, 5 days).
After inoculation and curing for 4 days (just before heating) this count had increased to 7.0 ± 0.1 log cfu/g (BHI, 20°C, 5 days). This agrees with the theoretical calculation of the inoculum.

Inoculum batch 2

The fresh meat used for production of hams for RF-cooking had an initial count of 2.8 ± 0.2 log cfu/g (BHI, 20°C, 5 days).
After inoculation and curing for 7 days (just before heating) this count had increased to 7.8 ± 0.2 log cfu/g (BHI, 20°C, 5 days). This number of bacteria is approx. 1 log higher than expected from the theoretical calculation of the inoculum. One explanation might be growth during the 7 days of curing/storage.

Curing

The cured hams and the heated hams were analysed on several different substrates. The results are shown in Table 8 and 9.

The total count on BHI-agar (20°C/5 days) and lactic acid bacteria on MRS-agar (20°C/5 days) increased by 0.5 to 0.9 log cfu/g during curing.

The number of bacteria counted on Slanetz agar (enterococci) and STA-agar (brochotrix) did not change during curing.

Inactivation during heat treatment

Heat treatment

The temperature profile during heating is shown in Figure 2 and 3.

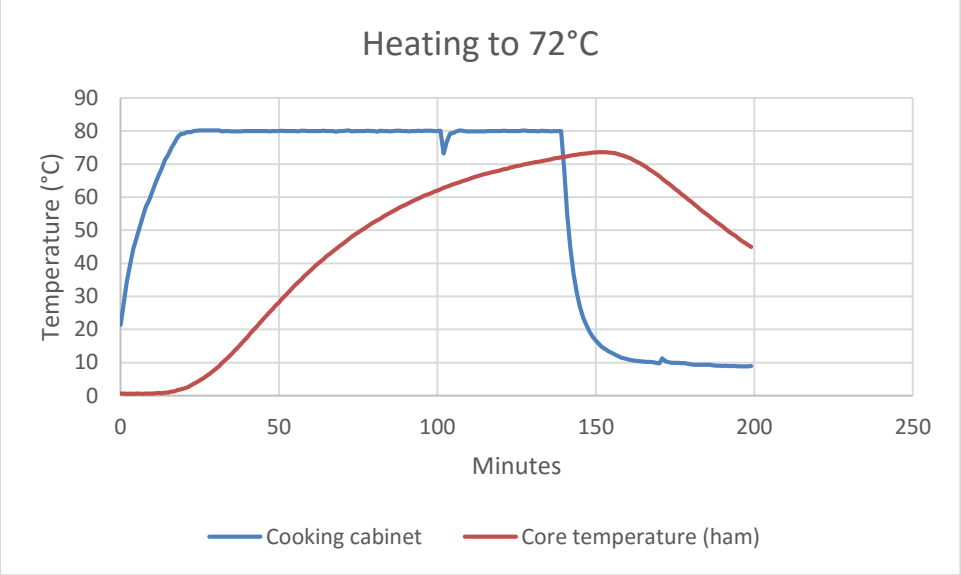


Figure 2. Heating to 72°C. F_{70}=105 minutes and F_{65}=716 minutes. T_{max}=73.6°C.

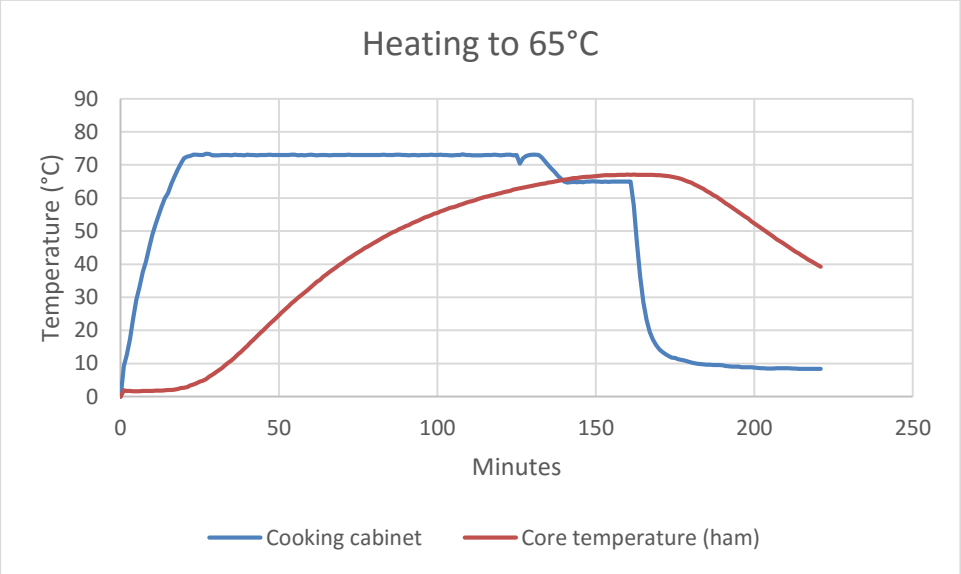


Figure 3. Heating to 65°C. F_{70}=13 minutes and F_{65}=93 minutes. T_{max}=67.1°C.

RF cooking

Hams S100, S101, S102, S103 and S105 received 380 kJ/kg for about 12 minutes.

Hams S106, S109, S110, S111 and S112 received 360 kJ/kg for about 12 minutes.

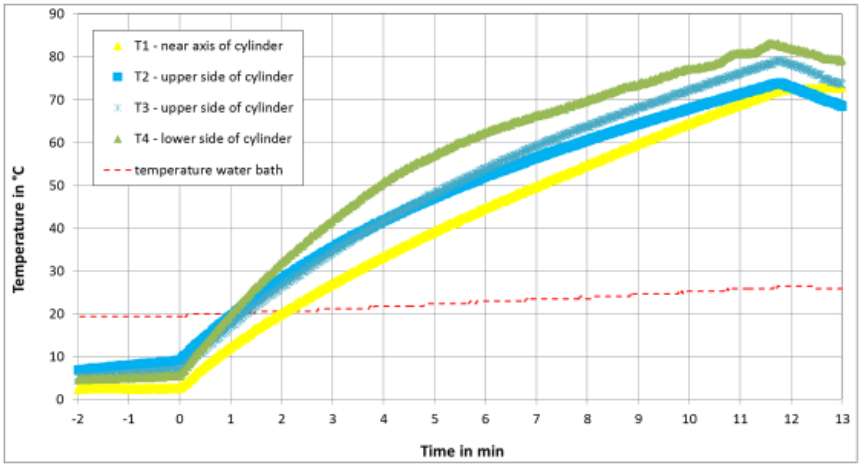
The water bath used during RF-cooking was approximately 20°C.

After RF-treatment, the hams were transferred to a hot water bath at 74°C for 10 minutes to cook the outside of the ham.

Cooling in air at room temperature for 5 minutes and in the ice-water for 20-60 minutes.

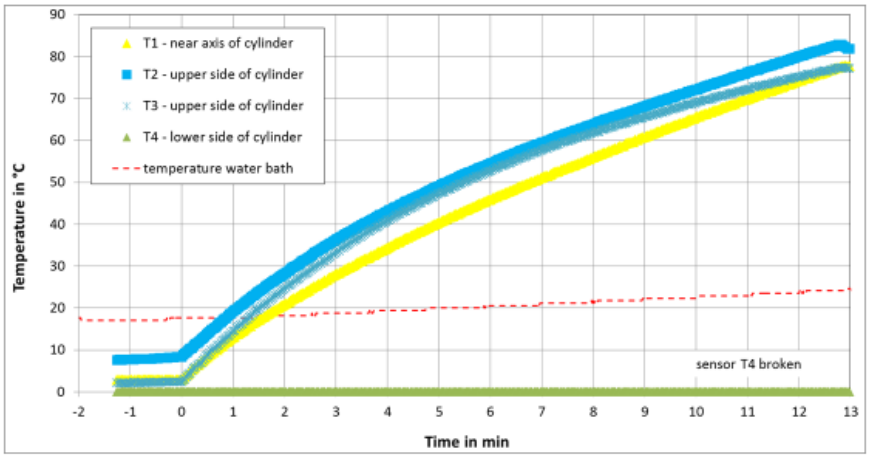
Storage at 1°C for 4 days before shipment to DMRI.

S108, fibre-optic measurement (360 kJ/kg)



Fraunhofer IVV

S107: fibre-optical temperature measurement (380 kJ/kg)



Fraunhofer IVV

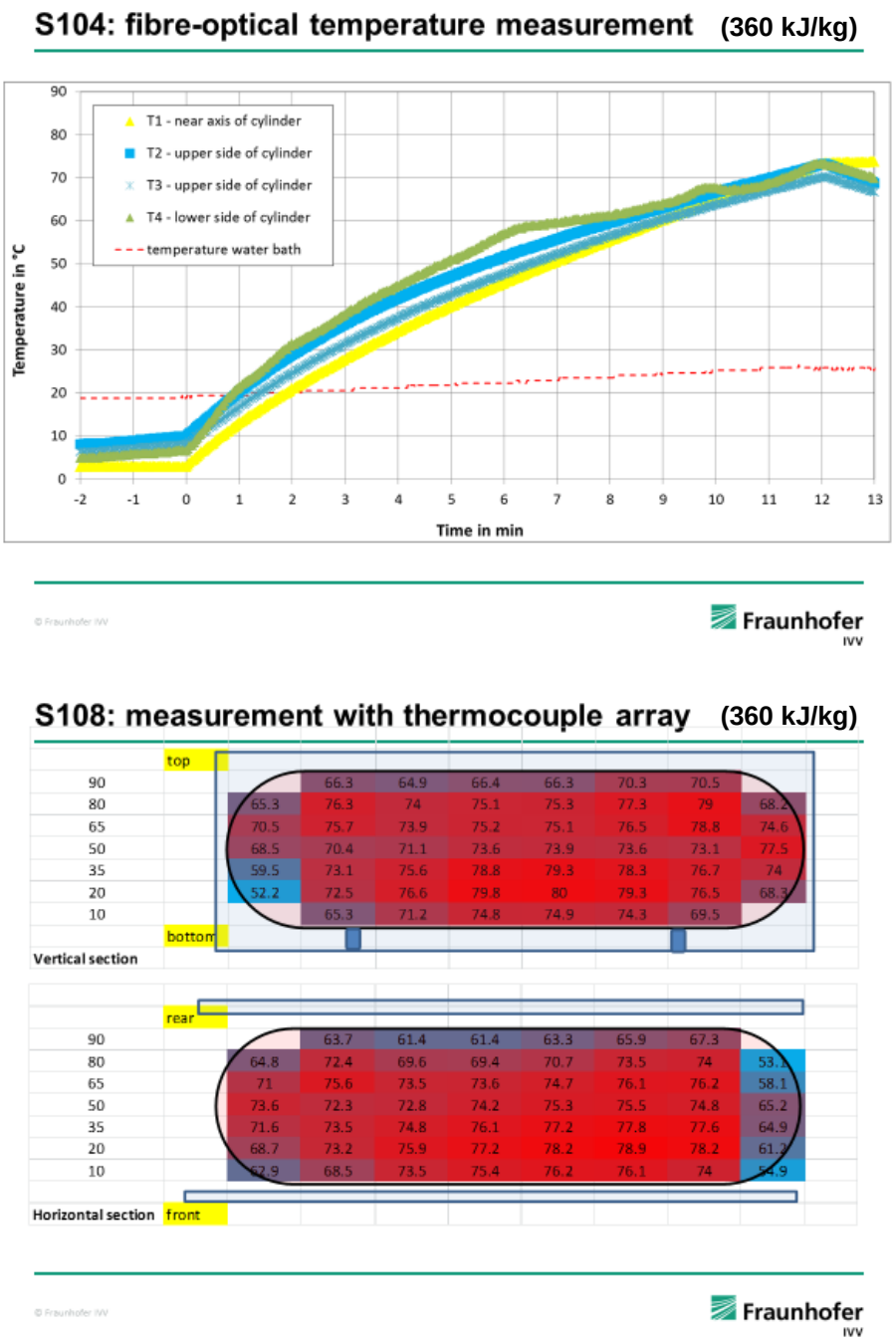


Figure 4. Temperature/time during RF-cooking. F_{70}-values for the microbial challenge test are not calculated. Cold spots are located close to the surface of the ham. After RF-cooking, the ham was heated in a water bath at 74°C. For further details, see Appendix 1.

Estimated reduction of bacteria

The measured D-values for the enterococci used in the challenge test and the data from the heat treatment were used to estimate the reduction of bacteria in the two traditional heat treatments. Furthermore, the reduction of pathogens like *salmonella* and *listeria* was estimated (Table 7).

The calculations in Table 7 show that none of the strains were expected to survive the heat treatment to 72°C whereas the heat resistant *E. faecium* strain used in the experiment should survive the heat treatment to 65°C as a reduction of only 2 log could be expected.

The calculation in Table 7 also shows that if the heat treatment used in the RF-cooking is capable of reducing the enterococci used in this experiment then the heat treatment used would be acceptable in order to inactivate vegetative cells of both spoilage and pathogens relevant to processed meat. Spores from *Bacillus* spp. and *Clostridium* spp. were not inactivated neither by traditional cooking to 72°C nor by RF-cooking to this temperature.

Table 7. Log reduction of bacteria at the used heat treatment to 72°C or 65°C.

Bacteria	D-value	Log reduction:	
		72°C (F_{70}=105)	65°C (F_{70}=13)
E. faecium	D_{70}=0.32[a)]	329	42
	D_{70}=1.73[a)]	60	8
	D_{68}=17[b)]	13	2
	D_{65}=55[b)]	13	2
E. faecalis	D_{65}=0.8[b)]	1546	125
	D_{60}=8[b)]	1547	125
Listeria	D_{70}=0.14[a)]	752	97
Salmonella	D_{70}=0.32[a)]	329	42

[a)] value from the literature (Koch, 2016), z=5
[b)] value measured in this project, z=5

Heating to 72°C

The heating to 72°C (F_{70}=105 minutes) inactivated all bacteria added to the cured ham including the ones proliferated during curing (Table 8).
In 3 out of 12 samples, 1 colony was detected which equals 10 cfu/g. This number was only detected on the non-selective agar, BHI at 37°C and 45°C, but not on the enterococci substrate, Slanetz. The detected colonies can be bacillus as their spores will survive heating to 72°C, or it might have been heat resistant enterococci protected in the ham. The most possible explanation is spores from *Bacillus* spp.

Heating to 65°C

The heating to 65°C (F_{70}=13 minutes) did not inactivate all bacteria added to the cured ham (Table 8).
The microbial dilution for counting on BHI-agar was too high meaning that the detection level was too high (3 log cfu/g). However, during sampling one week after heating, bacterial numbers at 2-3 log cfu/g were found. A new sampling from 65°C hams, stored at 2°C for 1 month showed that 2.6 log cfu/g had survived the heating when measured on the non-selective substrate BHI at 37°C whereas only <1-1.3 log cfu/g enterococci (Slanetz agar) had survived.
The survivors measured on BHI/37°C were not identified. They might be stressed enterococci or spores from *Bacillus* species.

The results show that some of the enterococci survived the heat treatment to 65°C (F_{70}=13). The lactic acid bacteria, *E. coli* and *Brochotrix* did not survive.

RF-cooking

The RF-cooking did not inactivate all bacteria added to the cured ham (Table 9). There were only small differences in the number of survivors when heating to 360 kJ and heating to 380 kJ. Survivors were detected on the non-selective substrate (BHI) incubated at 20°C, 37°C and 45°C and on MRS incubated at 20°C. No survivors were detected on Slanetz or STA-agar. Some of the isolated survivors measured on BHI/37°C were tested for growth on Slanetz agar and in 5 out of 8 samples tested, the survivors were shown to be enterococci. This shows that the enterococci are so stressed after the heat treatment that they are unable to grow at the selective substrate Slanetz at 45°C. Among the survivors measured on BHI-agar, other bacteria than enterococci might have been isolated, for example spores from *Bacillus* species.

Table 8. Microbial count (log cfu/g) before and after heating inoculated ham to 72°C or 65°C.

Substrate	Before stuffing (n=3)	Before heating (n=6)	Heating to 72°C (n=12)[1)]	Heating to 65°C (n=12)[1)]	Heating to 65°C (n=12)[2)]
BHI, 20°C/5 days	6.5 ± 0.2	7.0 ± 0.1	<1	<3-3.3[d)]	ND
BHI, 37°C/1 day	6.6 ± 0.1	6.9 ± 0.5	<1-1[a)]	<3-3[c)]	2.6 ± 0.6
BHI, 45°C/2 days	6.1 ± 0.1	5.8 ± 0.9	<1-1[a)]	<3	ND
E. coli petrifilm, 37°C/2 days	5.7 ± 0.1	5.1 ± 0.5	<1	<1	ND
Slanetz, 45°C/2 days (egg)	5.3 ± 0.1	5.4 ± 0.1	<1	<1-1.8[b)]	<1-1.3[e)]
Slanetz, 45°C/2 days (red)	5.2 ± 0.1	5.5 ± 0.1			
STA, 20°C/5 days	<4-4.5	<4-4.5		<1	ND
MRS-S, 20°C/5 days	6.3 ± 0.1	6.9 ± 0.1	<1	<1	ND

1) Heated to 65°C at DMRI and analysed 5 days after cooking. Three samples from each of four different hams.
2) Heated to 65°C at DMRI and stored at 2°C for 4 weeks before analysis. Three samples from each of four different hams.
a) 3 samples with 1 log cfu/g, 9 samples with <1 log cfu/g.
b) 1 sample with 1.8 log cfu/g, 11 samples with <1 log cfu/g.
c) 2 samples with 3 log cfu/g, 10 samples with <3 log cfu/g.
d) 1 sample with 3 log cfu/g, 1 sample with 3.3 log cfu/g, 10 samples with <3 log cfu/g.
e) 2 samples with 1 log cfu/g, 1 sample with 1.3 log cfu/g, and 9 samples with < 1 log cfu/g
ND: not analysed

Table 9. Microbial count (log cfu/g) in inoculated ham before and after RF-cooking

Substrate	Before stuffing (n=2)	Before heating (n=6)	RF-cooking at 360 kJ/kg (n=8)[1)]	RF-cooking at 380 kJ/kg (n=7)[1)]
BHI, 20°C/5 days	7.0 ± 0.0	7.8 ± 0.1	<1-1.7	<1-2.4
BHI, 37°C/1 day	7.0 ± 0.0	7.8 ± 0.1	2.4[d)] ±0.4	<1-3.1[c,d)]
BHI, 45°C/2 days	6.0 ± 0.0	6.2 ± 0.4	<1-1.5[b)]	<1-1.6[a)]
Slanetz, 45°C/2 days	5.5 ± 0.0	5.8 ± 0.2	<1	<1
STA, 20°C/5 days	4.7 ± 0.3	4.8 ± 0.2	<1	<1
MRS-S, 20°C/5 days	6.9 ± 0.0	7.8 ± 0.1	<1-1.6	<1-2.3

1) RF-cooked at Fraunhofer, analyzed 1 week after cooking (3 or 4 samples from each of two different hams). 360 kJ/kg (ham S106+S111); 380 kJ (ham S102+S105)
a) 4 samples <1 log cfu/g; 2 samples: 1 log cfu/g; 1 sample: 1.6 log cfu/g
b) 4 samples <1 log cfu/g; 2 samples: 1 log cfu/g; 1 sample: 1.3 log cfu/g; 1 sample: 1.5 log cfu/g
c) 3 samples <1; 1 sample: 1.3 log cfu/g; 1 sample: 1.3 log cfu/g; 1 sample: 2.1 log cfu/g, 2 samples: 3.1 log cfu/g.
d) Single colonies were streaked on the surface of Slanetz-agar at 45°C. They grew with typical red colonies indicating that they belong to the faecal enterococci.

Microbial growth of survivors during 2 months of storage

Samples for microbial analysis after 2 months of storage

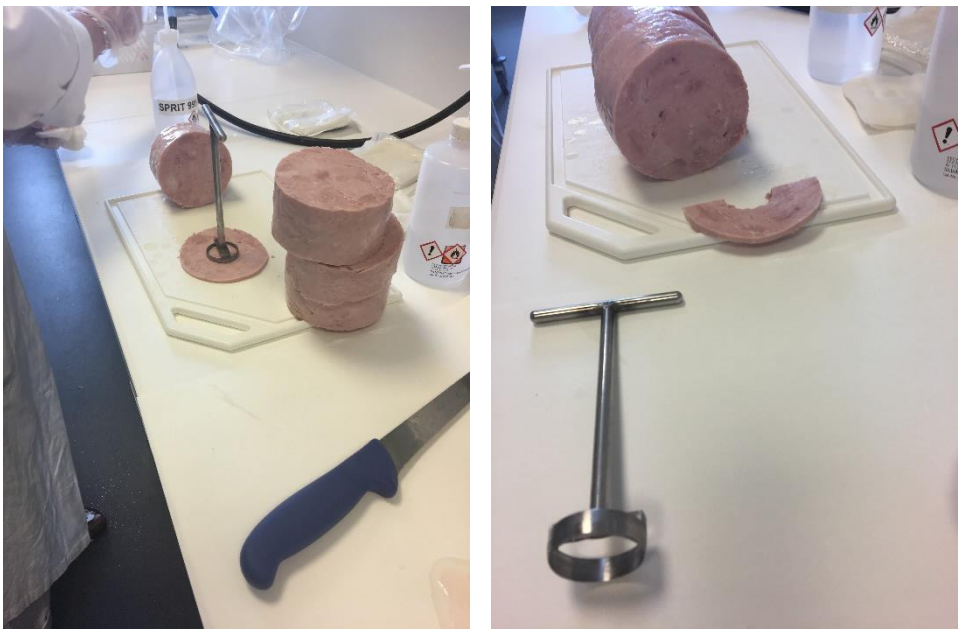


Figure 5. Sampling for microbial analysis of survivors in the core or the outer part of a ham slice.

Growth during storage at 5°C

The hams (all cooked in impermeable casing) were stored unbroken at 5°C for 2 months and then analysed on the same substrates as after heating (Table 10). For each combination of heat treatment and storage temperature, three hams were analysed. From each ham, 4 slices of ½-1 cm were analysed. Furthermore, RF-cooked hams were investigated for survivors in the core and in the other part of 5 slices of each ham (se Figure 5).

In all hams cooked to 72°C, the number of survivors after heat treatment was less than 1 log cfu/g (Table 8). After 2 months of storage at 5°C this

number had increased to approx. 2 log cfu/g in some of the samples. In several samples, the number of bacteria was still below 1 log cfu/g (Table 10). This slight increase in numbers might be due to slowly growing bacteria surviving the heat treatment or to heat injured cells not countable just after heat treatment. The traditional plate counting did not indicate what kind of bacteria that did survive heating and were countable in low numbers after 2 months of storage at 5°C. None of the samples were used for 16S sequencing as the number of bacteria was below the reliable number for sequencing (3-4 log cfu/g) in the set-up at DMRI.

In hams cooked to 65°C, the number of survivors after heat treatment was approx. 3 log cfu/g. After 2 months of storage at 5°C, this number had increased to 5-7 log cfu/g in several samples (in 3 of the 12 samples the number was only 2 log cfu/g) (Table 10). This indicates that bacteria have survived the heat treatment to 65°C/30 minutes (F_{70}=13 minutes) and been able to grow in the ham during storage at 5°C for 2 months. The traditional plate counting indicates that some of the survivors belong to enterococci, but other strains also seem to be part of the microflora that did survive and afterwards initiated growth during storage at 5°C. Nine samples were analysed by 16S sequencing. The last 3 samples were not analysed as the number of bacteria detected was below 3 log cfu/g. The results of sequencing are discussed later (Table 15).

In RF-cooked hams, only one ham (S110) cooked at 360 kJ showed survivors with numbers above 3 log cfu/g after 2 months of storage (Table 10). The 16S sequencing of these 2 slices (outer part of slice 1 and outer part of slice 5) are discussed later (Table 18).

Table 10. Microbial count (log cfu/g) in ham stored at 5°C for 2 months. An entire slice was analysed (4 slices from 3 different hams).

Substrate	72°C cooking (n=12)	65°C cooking (n=12)	RF[f] cooking (n=12)
BHI, 20°C/5 days	<1-2.5[e]	5.6±2.1	<2
BHI, 37°C/1 day	<1-1.8[d]	5.4±2.1	<2-2[g]
BHI, 45°C/2 days	<1-1.6[b]	<1-6.5[c]	<2-2[h]
Slanetz, 45°C/2 days	<1	<2-3.5[a]	<2
STA, 20°C/5 days	<1	<2	<2
MRS-S, 20°C/5 days	<1	<2	<1

[a] 6 samples <2 log cfu/g; mean of 6 samples: 3.0 ± 0.6 log cfu/g
[b] 10 samples <1 log cfu/g; 1 sample 1.0 log cfu/g; 1 sample 1.6 log cfu/g
[c] 7 samples <1 log cfu/g, mean of 5 samples: 4.5 ± 1.9 log cfu/g
[d] 4 samples <1 log cfu/g, mean of 8 samples: 1.5 ± 0.3 log cfu/g
[e] 2 samples <1 log cfu/g, mean of 10 samples: 1.5 ± 0.5 log cfu/g
[f] RF-cooking at 360 kJ or 380 kJ.
[g] 9 samples <2 log cfu/g; 3 samples 2.0 log cfu/g
[h] 11 samples <2 log cfu/g; 1 sample 2.0 log cfu/g

Growth during storage at 8°C

The hams (all cooked in impermeable casing) were stored unbroken at 8°C for 2 months and then analysed on the same substrates as after heating (Table 11).

In all hams cooked to 72°C, the number of survivors after heat treatment was less than 1 log cfu/g (Table 8). After 2 months of storage at 8°C, this number had increased to approx. 2 log cfu/g in some of the samples. In several samples, the number of bacteria was still below 1 log cfu/g (Table 11). This slight increase might be due to slowly growing bacteria surviving the heat treatment or to heat injured cells not countable just after heat treatment. The traditional plate counting did not indicate what kind of bacteria that did survive and afterwards initiated growth during storage at 8°C. Nine of the 12 samples were used for 16S sequencing as the number of bacteria was approx. 3 log cfu/g. This is the lowest number suitable for 16S sequencing in the set-up at DMRI now. The results are discussed later (Table 17).

In hams cooked to 65°C, the number of survivors after heat treatment was approx. 3 log cfu/g. After 2 months of storage at 8°C, this number had increased to 6-8 log cfu/g (Table 11). This indicates that bacteria have survived the heat treatment to 65°C/30 minutes (F_{70}=13 minutes) and been able to grow in the ham during storage at 8°C for 2 months. The traditional plate counting indicates that some of the survivors belong to enterococci, but other strains also seem to be part of the microflora that did survive and afterwards initiated growth during storage at 8°C. All 12 samples were analysed by 16S sequencing. The results are discussed later (Table 16).

In RF-cooked hams stored at 8°C for 2 months only one ham (S101) cooked at 380 kJ showed survivors with numbers close to 3 log cfu/g (Table 11). The count was found in the gel/exudate from ham S101. The results from 16S sequencing are discussed later (Table 18).

Table 11. Microbial count (log cfu/g) in ham stored at 8°C for 2 months. An entire slice was analysed (4 slices from 3 different hams).

Substrate	72°C cooking (n=12)	65°C cooking (n=12)	RF[c)] cooking (n=12)
BHI, 20°C/5 days	2.8±0.5	7.8±0.9	<2
BHI, 37°C/1 day	2.6±0.5	7.8±0.7	<2-2[d)]
BHI, 45°C/2 days	<1-1.5[b)]	6.4±1.3	<2-2[d)]
Slanetz, 45°C/2 days	<1	<2-3.5[a)]	<2
STA, 20°C/5 days	<1	<2	<2
MRS-S, 20°C/5 days	<1	<2	<1

[a)] 2 samples: <2 log cfu/g; mean of ten samples: 3.1 ± 0.4 log cfu/g
[b)] 8 samples <1 log cfu/g; 3 samples 1.0 log cfu/g; 1 sample 1.5 log cfu/g
[c)] RF-cooking at 360 kJ or 380 kJ.
[d)] 11 samples <2 log cfu/g; 1 sample 2.0 log cfu/g

Cold spots in RF-cooked ham

To investigate if cold spots occurred in the RF-cooked ham, five new slices were made from each ham. These slices were divided into three samples, 1 core sample and 2 outer samples (Figure 5).

Only 1 ham (2 separate slices) had microbial counts above 2-2.5 log cfu/g. In 2 out of 9 slices, survivors were detected (it cannot be ruled out whether the detected bacteria are only survivors or if they also have multiplied during storage). The cold spot was located in the outer part of the ham. These samples are shown in Table 12. All samples are summarized in Table 13 and 14. This shows that only a few microorganisms were detected in all the samples analysed indicating that the heat treatment during RF-cooking has been efficient in inactivating the added bacteria except for on ham cooked at only 360 kJ.

Table 12. Microbial count (log cfu/g) in ham S110, RF-cooked at 360 kJ + post pasteurized and stored at 5°C for 2 months. Outer part and core analysed separately (n=1).

	Sampling place in S110						
	Slice 1			Slice 5			Gel
Substrate	Core	Out 1	Out 2	Core	Out 1	Out 2	
BHI, 20°C/5 days	<2	<2	2.5	<2	<2	4.2	<2
BHI, 37°C/1 day	<2	<2	3.1	<2	2.5	4.2	<2
BHI, 45°C/2 days	<2	<2	<2	<2	<2	4.0	<2
Slanetz, 45°C/2 days	<2	<2	<2	<2	<2	2.3	<2
STA, 20°C/5 days	<2	<2	<2	<2	<2	<2	<2
MRS-S, 20°C/5 days	<1	<1	<1	<1	<1	<1	<1

Table 13. Microbial count (log cfu/g) in RF-cooked ham stored at 5°C for 2 months. Outer part and core analysed separately. In total, 15 slices were analysed (3 samples each slice).

	360 kJ (ham S109+S110, 5 slices each)			380 kJ (ham S100, 5 slices)		
Substrate	Core	Outer 1	Outer 2	Core	Outer 1	Outer 2
BHI, 20°C/5 days	<2-2[a)]	<2	<2-4.2[d)]	<2	<2	<2
BHI, 37°C/1 day	<2	<2-2.5[a)]	<2-4.2[c)]	<2	<2-2[a)]	<2[a)]
BHI, 45°C/2 days	<2	<2	<2-4.0[b)]	<2	<2	<2-2[a)]
Slanetz, 45°C/2 days	<2	<2	<2-2.3[a)]	<2	<2	<2
STA, 20°C/5 days	<2	<2	<2	<2	<2	<2
MRS-S, 20°C/5 days	<1	<1	<1	<1	<1	<1

a) Only one sample, all other <2.
b) 1 sample 4.0; 1 sample 2.0; all other <2.
c) 1 sample 4.2; 1 sample 3.1; 1 sample 2.3; 1 sample 2.0, all other <2.
d) 1 sample 4.2; 1 sample 2.5; 1 sample 2.0; all other <2.

360 kJ/kg (ham S109+S110); 380 kJ (ham S100)

Table 14. Microbial count (log cfu/g) in RF-cooked ham stored at 8°C for 2 months. Outer part and core analysed separately. In total, 15 slices were analysed (3 samples of each slice)

Substrate	360 kJ (ham S112, 5 slices)			380 kJ (ham S101+S103, 5 slices each)		
	Core	Outer 1	Outer 2	Core	Outer 1	Outer 2
BHI, 20°C/5 days	<2	<2	<2	<2	<2-2[a)]	<2
BHI, 37°C/1 day	<2	<2-2.3[a)]	<2	<2	<2-2.3[a)]	<2-2[b)]
BHI, 45°C/2 days	<2	<2	<2	<2	<2	<2-2[b)]
Slanetz, 45°C/2 days	<2	<2	<2	<2	<2	<2
STA, 20°C/5 days	<2	<2	<2	<2	<2	<2
MRS-S, 20°C/5 days	<1	<1	<1	<1	<1	<1

a) 1 sample, all other <2

b) 2 samples, all other <2

360 kJ/kg (ham S112); 380kJ (ham S101+S103)

Microbiota in hams stored for 2 months at 5°C and 8°C

Samples with 3-8 log cfu/g were chosen for characterization of the microbiota by 16S sequencing.

Qiagen DNeasy Blood and Tissue kit (cat no. 69504) was used for DNA extraction. The ds-DNA concentration was measured with Qubit 3.0.

A 16 S library of DNA from each sample was made in two steps. PCR 1 (amplicon PCR, forward primer 5'-TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG CCT ACG GGN GGC WGC AG, reverse primer 5'-GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GGA CTA HVG GGT ATC TAA TCC, overhang indicated in yellow) and PCR 2 (index PCR, using NexteraXT Index Primer 1 (N7xx) and Primer 2 (S5xx)).

Fragment analyser was used to measure the size and concentration of DNA fragments. The HS NGS Fragment analyser kit, 1 – 6000 bp from Kem-En-Tek was used.

The PCR products were purified after PCR 1 and PCR 2.

The size of PCR products after PCR 2 were approx. 600 bp. The size of the locus specific sequence was 465 bp.

Sequencing was performed on an Illumina MiSeq, and the protocol by Illumina was used.

The sequence data was processed on BION software ver. 16.12 (Danish Genome Institute).

In Table 15-19, the identification of the survivors causing growth in the hams during storage at 5°C and 8°C for 2 months is shown.

The results (Table 15) from 16S sequencing indicate that the survivors from heating to 65°C initiating growth at 5°C were dominated by *E. faecium* in two hams (2-1, 2-2). In ham 2-3, *E. faecium, E. faecalis* and *Lb. sakei* dominated. The number of bacteria in this ham was a little lower than in ham 2-1 and 2-2. This might be an explanation for the more diverse microbiota. The reason is that if a culture is present in a concentration of 5 log cfu/g then it might be difficult to find by 16S sequencing if another strain is present in a number of 7 log cfu/g as it will only count for 1% of the microbiota.

The results (Table 16) indicate that the survivors from heating to 65°C initiating growth at 8°C were dominated by *E. faecium*. In a few samples (ham 2-1, slice 1 and ham 2-3 slice 1) *Lb. sakei* was also found in high numbers of the reads. The total count (BHI/37°C) was approx. 1 log lower in the samples where *Lb. sakei* was part of the domination microbiota.

The results (Table 17) indicate that the few survivors from heating to 72°C initiating growth at 8°C were dominated by *Lb. sakei*. Furthermore, the 16S sequencing shows that *Brochotrix, E. faecalis* and *E. faecium* were also among the dominating reads. Further research will have to investigate whether these findings are due to survival and possible growth of these organisms OR if these findings are partly due to small DNA fragments from bacteria inactivated in the heating process. It must be noted that a high number of the bacteria represented by the detected sequences were added to the ham before heating.

The results (Table 18) indicate that the survivors in one cold spot in RF-cooked ham initiating slow growth at 5°C were dominated by *Lb. sakei* and *E. faecium.* However, like in the samples from hams heated to 72°C (Table 17), these RF-cooked samples also showed reads from *Brochotrix* and *E. faecalis* indicating that these organisms were among the dominating microbiota in the samples. Further research will have to investigate whether these findings are due to survival and possible growth of these organisms OR if these findings are partly due to small DNA fragments from bacteria inactivated in the heating process. It must be noted that a high number of the bacteria represented by the detected sequences were added to the ham before heating.

The results show that survival and growth are caused by the microorganisms added. No background microbiota from the used meat or ingredients were detected.

When ham was heated to only 65°C, *E. faecium* were the dominating species initiating growth during storage at 5°C and 8°C during 2 months (the ham was not sliced after heat treatment but stored in the non-permeable casing from the cooking process) (Table 15 and 16).

When ham was heated to 72°C, *Lb. sakei* became the dominating species detected by 16S sequencing after 2 months of storage at 8°C. However, further research is needed to verify if this is survivors with slow growth or if the result is due to small DNA fragments (Table 17).

When ham was RF-cooked to 72-74°C, a few samples showed survivors with 2-3 log cfu/g. The 16S sequencing indicates that *Lb. sakei* is the dominating species but also *Brochotrix, E. faecalis* and *E. faecium* are among the dominating reads. Further research is needed to verify if this is survivors with slow growth or if the result is due to small DNA fragments (Table 18).

Table 15. Number of bacteria (BHI, 37°C, log cfu/g) and results from 16S sequencing. Ham cooked to 65°C and stored at 5°C for 2 months.

			Percent of analysed sequences			
Ham	Slice	Log cfu/g	*Brochotrix*	*E. faecalis*	*E. faecium*	*Lb. sakei*
2-1	2[a)]	6.7	0	1	90	8
2-1	3	7.0	0	0	97	2
2-1	4	7.0	0	0	98	2
2-2	2	7.0	0	0	97	3
2-2	3	7.0	0	0	97	2
2-2	4[b)]	7.0	0	0	97	2
2-3	2	5.0	8	22	0	70
2-3	3	6.6	1	1	78	20
2-3	4[c)]	5.7	2	3	46	45

97-100% of reads mapped to unic species were used.
[a)] furthermore 1% *E. coli* was measured
[b)] furthermore 1% *C. maltaromaticum* was measured
[c)] furthermore 3% *E. coli* was measured

Table 16. Number of bacteria (BHI, 37°C, log cfu/g) and results from 16S sequencing. Ham cooked to 65°C and stored at 8°C for 2 months.

			Percent of analysed sequences			
Ham	Slice	Log cfu/g	*Brochotrix*	*E. faecalis*	*E. faecium*	*Lb. sakei*
2-1	1	6.4	3	4	41	50
2-1	2	8.2	0	0	98	1
2-1	3	8.3	0	0	99	1
2-1	4	8.2	0	0	97	2
2-2	1	6.9	1	0	93	6
2-2	2	8.2	0	0	98	2
2-2	3	8.4	0	0	98	2
2-2	4	8.0	0	0	98	2
2-3	1	6.9	0	1	78	19
2-3	2	8.3	0	0	99	1
2-3	3	8.1	0	0	98	2
2-3	4	8.2	0	0	98	2

98-100% of reads mapped to unic species were used.

Table 17. Number of bacteria (BHI, 37°C, log cfu/g) and results from 16S sequencing. Ham cooked to 72°C and stored at 8°C for 2 months.

			Percent of analysed sequences			
Ham	Slice	Log cfu/g	*Brochotrix*	*E. faecalis*	*E. faecium*	*Lb. sakei*
1-1	1	3.0	5	5	8	73
1-1	2	3.2	5	4	7	78
1-1	3	2.9	5	5	0	75
1-1	4	3.0	5	5	8	75
1-2	1	3.0	6	6	0	69
1-3	1	2.9[a)]	7	8	0	74
1-3	2	3.2[a)]	6	5	11	74
1-3	3	3.1[a)]	0	0	25	53
1-3	4	2.9[a)]	5	5	8	75

92-98% of reads mapped to unic species were used. Showing that many different sequences were obtained, but each counting for less than 0.5%. This is probably due to a low number of viable bacteria.

[a)] BHI, 20°C/5 days.

Table 18. Number of bacteria (BHI, 37°C, log cfu/g) and results from 16S sequencing. Ham before and after RF-cooking at Fraunhofer to 72°C and post-pasteurized at 74°C. No storage (only chilled transport from Fraunhofer to DMRI).

Process step	Ham	Slice	Log cfu/g	Percent of analysed sequences			
				Brochotrix	*E. faecalis*	*E. faecium*	*Lb. sakei*
No RF	1	-	8.0	1	2	1	96
	1x	-	7.6	2	0	47	51
	1xx	-	8.0	1	0	0	99
360 kJ	3-1	-	3.1	6	3	7	84
	3-1	x	3.1	10	5	14	71
	3-2	-	3.0	8	3	8	81
	3-2	x	2.6	9	3	10	77
	3-2	xx	2.5	5	2	6	87
380 kJ	3-3	x	2.2	6	4	6	84
	3-3	xx	2.0	7	4	10	79

99-100% of reads mapped to unic species were used. Showing that only a few different sequences were obtained even though the number of viable bacteria is low in several samples.

Table 19. Number of bacteria (BHI, 37°C, log cfu/g) and results from 16S sequencing. Ham RF-cooked at Fraunhofer to 72°C and post-pasteurized at 74°C and then stored at 5°C or 8°C for 2 months.

Temp. (°C)	Ham	Slice	Log cfu/g	Percent of analysed sequences			
				Brochotrix	*E. faecalis*	*E. faecium*	*Lb. sakei*
5	3-1	1-C	3.1	4	2	10	83
5	3-1	5-C	4.2	4	2	5	89
8	3-5	gel	2.8	18	14	14	53

99-100% of reads mapped to unic species were used. Showing that only a few different sequences were obtained even though the number of viable bacteria is low in several samples.
3-1 = S110; 3-5= S101; 1-C and 5-C (outer region of the slice)

Table 20. 16S sequencing of pure cultures (grown in BHI).

Bacteria	DMRICC	ID by MiSeq
E. faecium	4266	*E. faecium*
E. faecalis	4168	*E. faecalis*
E. durans	4371	*E. faecium; Lb. sakei*
B. termospachta	4738	*B. termospachta*
Lb. sakei	3852	*Lb. sakei*
S. thermophilus	5010	*S. salivarius*

Discussion

Survivors from the different heat treatments

All three heating programs reduced the amount of bacteria added to the ham. Heating to 65°C reduced the number to approx. 3 log cfu/g. Heating to 72°C reduced the number to <1-1 log cfu/g, and RF-cooking to 72-74°C reduced the number to <1-2 log cfu/g.

Growth of survivors

In our experiment, *E. faecium* and *Lb. sakei* surviving the heating to 65°C for 30 minutes (F_{70}=13) increased by 2-4 log in ham stored at 5°C and by 3-5 log in ham stored at 8°C for 2 months. This observation differs from the growth studies made by Zanoni et al. (1993) and Cermak et al. (2009).

Zanoni et al. (1993) have investigated the growth of *E. faecium* in mortadella (pH 5.6; NaCl 3.1%; moisture 61.69%) at temperatures from 5°C to 50°C. Growth at 5°C was very slow whereas at 12°C the maximum number would be reached within 10 days.

Using the model developed by Cermak et al. (2009), a 6 log growth can be expected at 5°C (Aw 0.97; pH 6) in 25 days.

In our experiment, hams heated to 72°C (traditional cooking) or RF-cooked to 72-74°C also showed an increased number of bacteria in a few samples after storage at 8°C for 2 months. The increase in detected numbers might be due to growth from a few survivors OR recovery of heat injured cells. Interestingly, the microbiota from these samples (both traditionally heating and RF-cooking) were dominated by *Lb. sakei* followed by *E. faecium.* This is remarkable as *Lb. sakei* has a much lower D-value than *E. faecium.*

Further research will have to investigate if *Lb. sakei* is able to survive pasteurization of processed meat. Sometimes researchers have discussed the possibilities of lactobacilli to survive traditionally heating regimes.

Cooking loss/Yield

Cooking loss was measured and showed the lowest cook out in ham cooked to 65°C/30 minutes followed by cooking to 72°C/2 minutes (traditionally). The highest cook out was found in RF-cooked ham.

However, more research is needed to conclude if RF-cooking gives more cook out that traditionally cooking. What can be concluded is that the texture of cook out in RF-cooked ham is watery compared to ham cooked for a longer time to 65°C or 72°C. In these hams, the cook out has a jelly-like texture.

Conclusion
Because of technical problems with casings and inoculum, different batches of raw material have been used. One for traditional cooking to 65°C and 72°C and one for RF-cooking.

Traditional heating to 72°C for 2 minutes (F_{70}=105 minutes) efficiently inactivated the cocktail of bacteria added as well as the natural background flora in the raw meat.

Traditional heating to 65°C for 30 minutes (F_{70}=13 minutes) did not inactivate all bacteria in the cocktail added nor all bacteria from the natural background flora in the raw meat.

RF-cooking with 360 kJ and 380 kJ to 72°C did not inactivate all bacteria in the cocktail added nor all bacteria from the natural background flora in the raw meat.

After 2 months of storage at 5°C, survivors from the 65°C heat treatment increased to 5-7 log cfu/g. 16 S sequencing indicated that the growth was caused by *E. faecium.* In ham heated to 72°C, no growth or a slight increase in bacteria was observed. The number was too low for 16S sequencing. In RF-cooked hams, only one cold spot was detected. The survivors increasing to 4 log cfu/g were dominated by *Lb. sakei.*

After 2 months of storage at 8°C, survivors from the 65°C heat treatment increased to 6-8 log cfu/g. 16 S-sequencing indicated that the growth was caused by *Lb. sakei* and *E. faecium.* In ham heated to 72°C, a slight increase in bacteria was observed. Hams with approx. 3 log cfu/g were analysed by 16S sequencing. This shows that *Lb. sakei* dominated the microbiota. In RF-cooked hams, one ham cooked at 380 kJ showed 3 log cfu/g in the gel. The sequencing indicated that these organisms are dominated by *Lb. sakei*.

The cooking loss was higher in hams RF-cooked at 380 kJ compared to 360 kJ. The cooking loss was higher in hams cooked to 72°C compared to hams cooked to 65°C. The order of cooking loss was:

65°C (5.2%) < 72°C (7.7%) ≈ 360 kJ (8.4%) < 380 kJ (14.5%)

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Zanoni, B., Garzaroli, C., Anselmi, S. & Rondinini, G. (1993) Modelling the growth of *Enterococcus faecium* in bologna sausage. *Applied and Environmental microbiology* 59(10)3411-3417.

Appendix 1.

Details on the RF-cooking

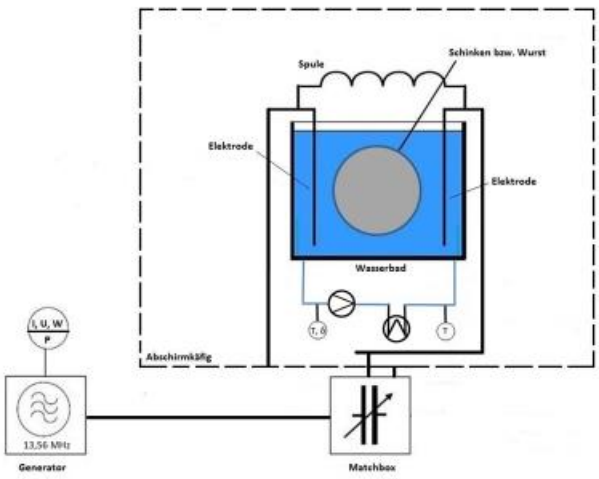
RF Cooking of Ham

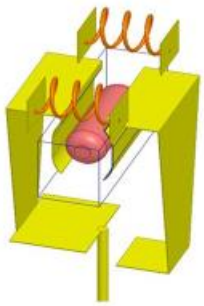
Experiment of April 28.,
Inoculated Samples of DMRI

Fraunhofer IVV

Thomas Pfeiffer
Fraunhofer-Institut für Verfahrenstechnik und Verpackung
Freising, May 12, 2017

RF-Ham cooking of samples for





[2]

Fraunhofer IVV

Experiment of April 28.: Inoculated Samples of DMRI

Sample number	weight in g	transferred energy in kJ	transferred energy in kJ/kg	heating time in min	RF voltage in V
S108	2934	1079	368	11.70	1400, 4 coils
S104	2980	1100	369	12.10	1400, 4 coils
S100	2960	1125	380	12.00	1400, 4 coils
S101	2970	1229	380	12.30	1400, 4 coils
S102	2918	1050	380	11.00	1400, 4 coils
S103	2966	1227	380	12.40	1400, 4 coils
S105	2973	1130	380	12.20	1400, 4 coils
S107	2962	1126	380	12.80	1400, 4 coils
S106	2966	1068	360	11.30	1400, 4 coils
S109	2959	1065	360	11.10	1400, 4 coils
S111	2963	1067	360	11.10	1400, 4 coils
S112	2958	1065	360	10.40	1400, 4 coils
S110	2941	1060	360	11.15	1400, 4 coils

Marked samples were used for tuning/checking of process, not treated in hot water bath!

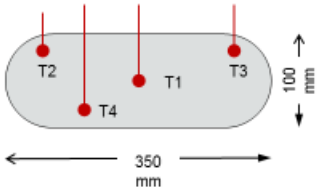
Fraunhofer IVV

Sample material

Hams: about 3 kg, 100 mm diameter, 350 mm length

Pork meat
- 15% brine injection
- 2% total salt

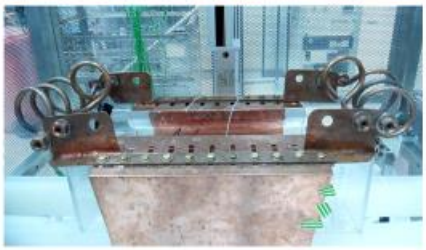
Start temperature near 1 °C!



Positions of fibre-optical sensors for inline measurement of temperatures,
sensor T1 at axis of ham,
sensors T2, T3, T4 about 10 mm from surface

Fraunhofer IVV

Water bath – top view

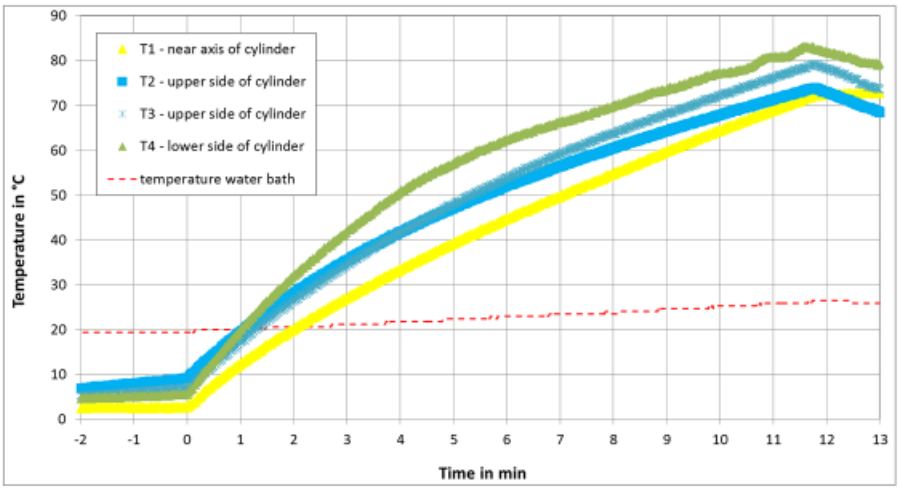


Water bath – front view

Ham to be equipped with fibre-optical sensors

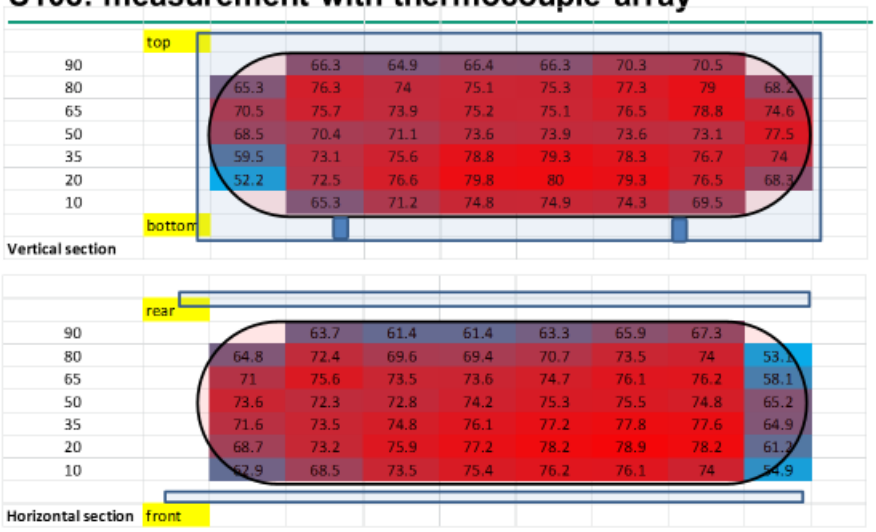
Fraunhofer IVV

S108, fibre-optic measurement



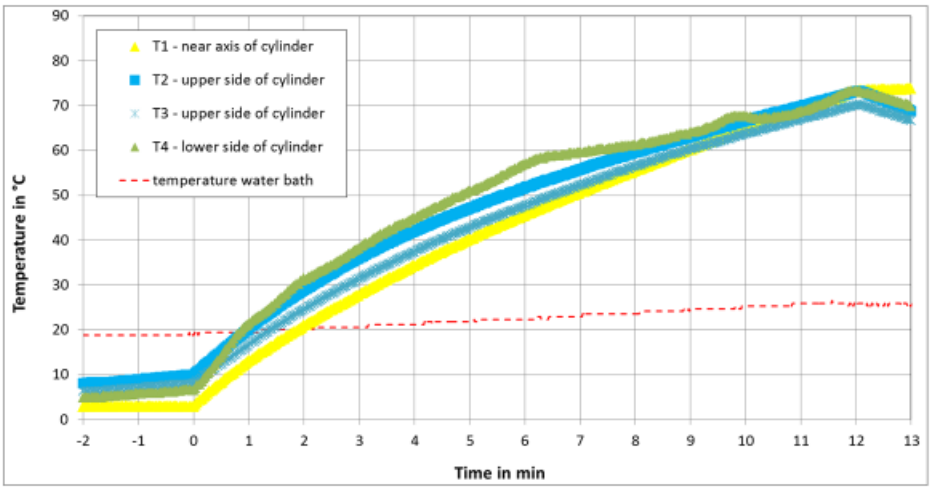
Fraunhofer IVV

S108: measurement with thermocouple array



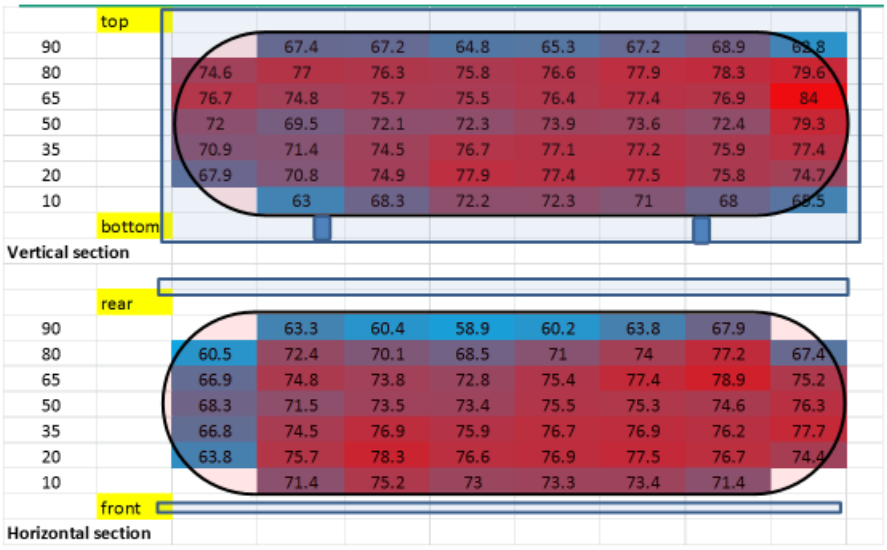
Fraunhofer IVV

S104: fibre-optical temperature measurement



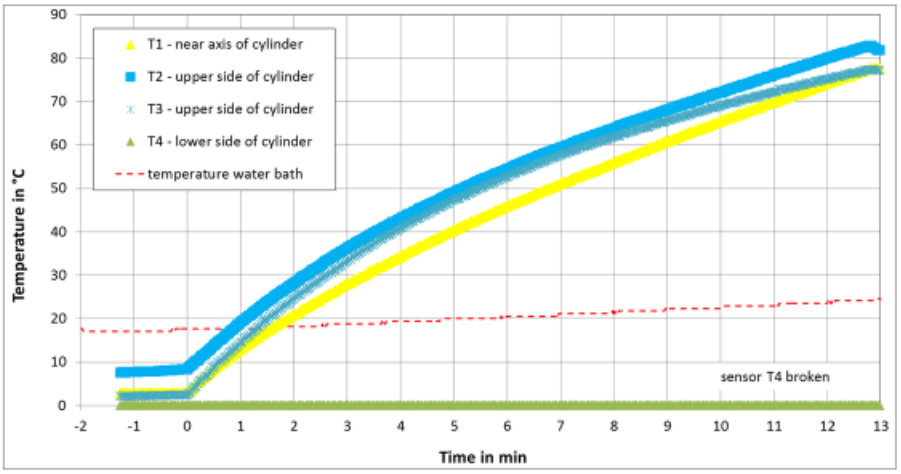
Fraunhofer IVV

S104: measurement with thermocouple array



Fraunhofer IVV

S107: fibre-optical temperature measurement



Fraunhofer IVV

S107: measurement with thermocouple array

	top							
90		70.7	71	62.1	66.4	67.4	71.7	67.1
80	72.5	84.8	80.3	77.6	78.5	79.8	82.8	78.6
65	84.2	82.9	80.7	80.3	80.1	80.5	82.3	83.6
50	83.9	76.9	77.2	78	77.2	76.7	76.7	85.2
35	72.2	77.9	80	80.2	79.3	78.9	78	77.3
20	60.9	75.6	78.9	79.4	79	78.5	76.3	69.4
10		64.2	70.2	71.9	74.3	71.5	66.4	
	bottom							

Vertical section

	rear							
90		65.7	62.8	60.4	61.3	63	65.1	56.2
80	50.1	77.7	75.9	73.3	74	75.1	76.1	69.9
65	53.9	80.4	80.2	78.4	77.9	78.9	79.9	78.4
50	56	78.8	78.7	78.7	78	77.6	77.7	83.8
35	51.7	80.9	79.8	79.9	79.8	80.3	81.2	84
20		80.4	80.3	79.7	79.8	80.5	81.5	80.3
10		73.2	76	74.5	74.6	74.5	74	70.8
	front							

Horizontal section

Fraunhofer IVV