DANISH MEAT
RESEARCH INSTITUTE

Report

RF cooking of ham

Hazard analysis

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Summary

Aim

The aim of this deliverable is to perform an analysis of the microbiological risk of the RF cooking process.

The analysis of microbial risk in the RF process is based on the results from the challenge tests (task 4.2), the calculated requirement for appropriate heat inactivation (task 4.1) and the measurements on temperature distribution in WP3. The risk analysis will point out how the RF cooking can be used for heat treatment of ham with high food safety standards.

Results

CCP in heating ham is to reach a temperature of 72°C in the entire product.

RF cooking at 380 kJ/kg meat results in a ham with a temperature between 50°C and 85°C. The coldest spots were in the outer ring of the ham. Therefore, this RF cooking process was combined with heating in a water bath at 74°C for 10 minutes to cook the outside of the ham.

RF cooking + heating in water bath did not inactivate all bacteria in the ham.

Survivors could increase in numbers during storage at 5°C and 8°C for 2 months.

Conclusion

Because of the detected cold spots and the results showing that some bacteria strains survived in a few spots, it is concluded that the RF process must be improved to result in a uniform heat treatment of the entire ham.

Furthermore, more research is also needed to investigate how *Lb. sakei* can survive RF cooking and traditional heating to 72°C in a cooking cabinet. It might be speculated how these bacteria are capable of protection against heat. Maybe like spore formers, or how they are introduced to the product after heating.

Introduction

Aim

The aim of this deliverable is to perform an analysis of the microbiological risk of the RF cooking process.

The analysis of microbial risk in the RF process is based on the results from the challenge tests (task 4.2), the calculated requirement for appropriate heat inactivation (task 4.1) and the measurements on temperature distribution in WP3. The risk analysis will point out how the RF cooking can be used for heat treatment of ham with high food safety standards OR what is needed to further develop the process for RF cooking of ham.

Background

The World Health Organization (WHO) describes risk analysis as a process composed of three elements risk assessment, risk management and risk communication. *Risk assessment* includes using scientific information to describe the likelihood and magnitude of harm attributed to a specific hazard. *Risk management* includes all activities undertaken to control a hazard. *Risk communication* is the exchange of information and opinions about a hazard among concerned parties. Risk analysis is accomplished through the efforts of separate but integrated assessment, management and communication teams.
In this report, an analysis is made of microbial hazards and the inactivation obtained using the RF cooking. The analysis uses hazard analysis and results from the project.

Hazard analysis is the process of recognizing hazards that may arise from a system or its environment, documenting their unwanted consequences and analyzing their potential causes.

The objectives of a hazard analysis are to:

- **Identify hazards.** To determine the hazards and hazardous events of the equipment under control and the control system (in all modes of operation), for all reasonably foreseeable circumstances including fault conditions and misuse
- **Identify causes.** To analyse the event sequences leading to the hazardous events identified
- **Determine risks.** To analyse the risks associated with the hazardous events.

Hazard identification – critical control points (CCP)

Ham processing

Cooking to 72°C in the entire product is a CCP in cooking ham.

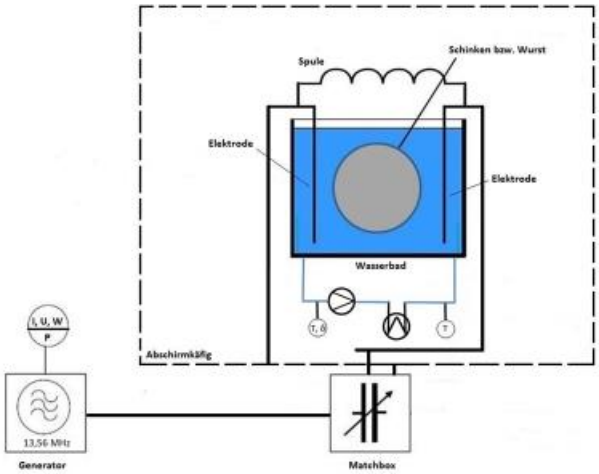
RF cooking

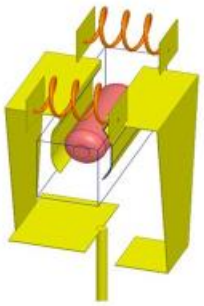
The RF heating process was combined with water immersion. This is different from prior attempts to cook ham or large meat parts in RF fields. In this test, the ham was kept sealed in a hermetic pouch during the process to prevent recontamination.

Furthermore, the packed ham was exposed to the RF field during immersion in a water bath, where the water acts as a transmitting medium for the electric field, providing a more uniform field inside the ham and avoiding overheating at edges and corners. Hence, the electrodes, which apply the field to the food, had been adapted to the shape and electric properties of the ham.

The critical control point is how the RF cooking performs in reaching the correct temperature in the entire product.

RF-Ham cooking of samples for





[2]

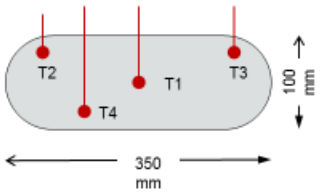
Fraunhofer IVV

Sample material

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

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

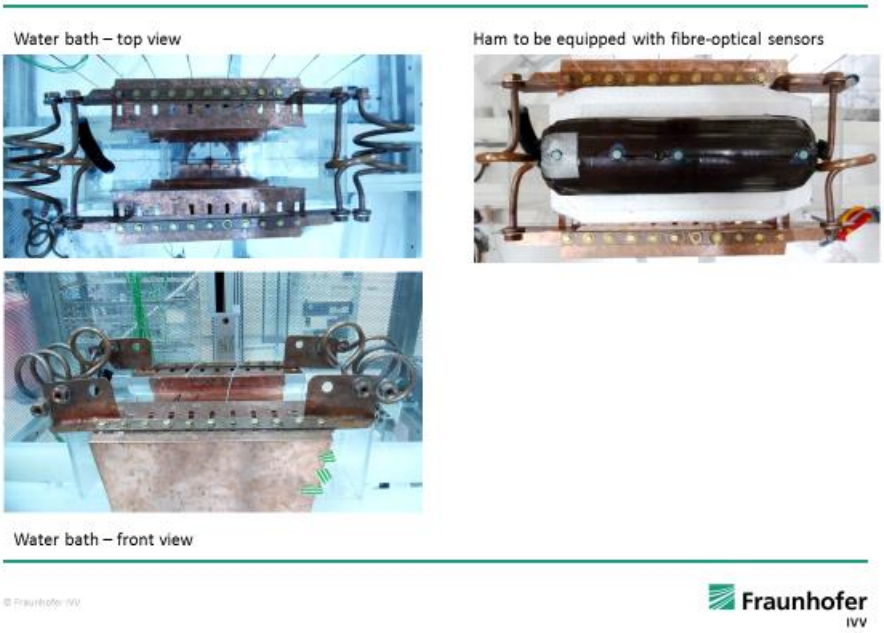
Start temperature near 1 °C!



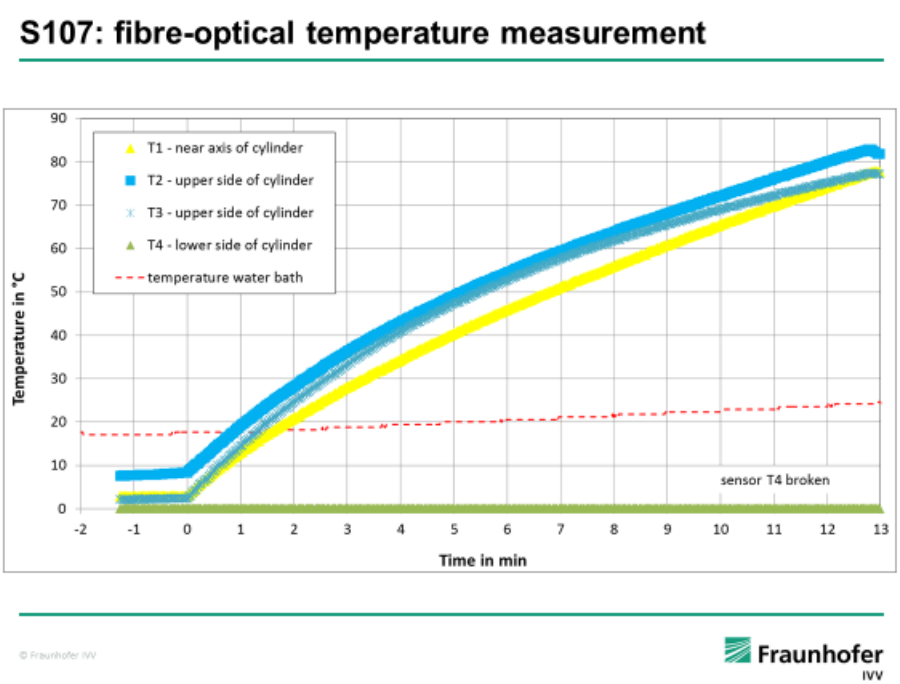
Positions of fibre-optical sensors for inline measurement of temperatures,
sensor T1 at axis of ham,
sensors T2, T3, T4 about 10 mm from surface

Fraunhofer IVV

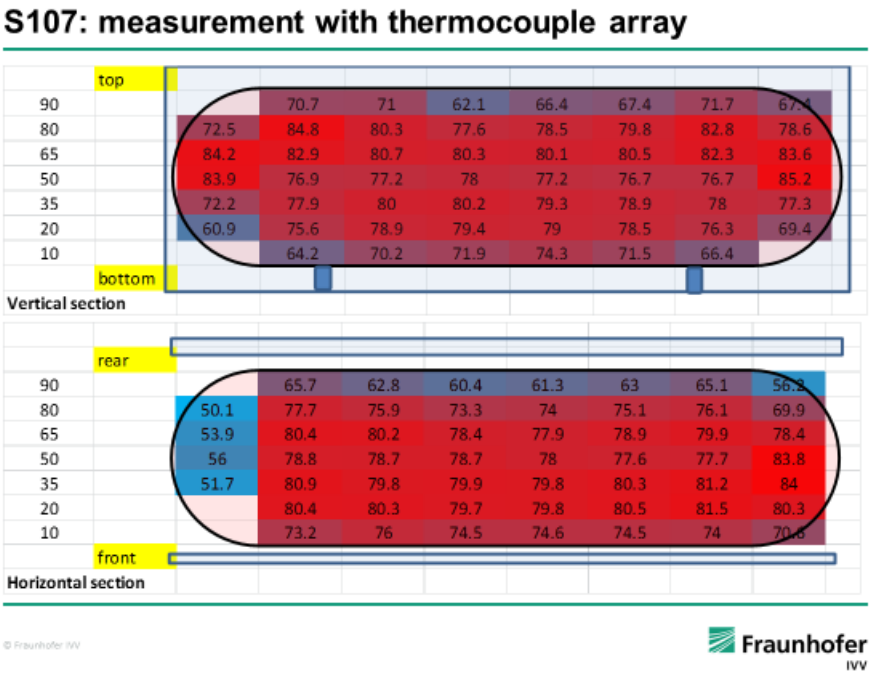
During heating, the temperature was measured in four places to ensure correct heating time and voltage.



The pictures show the experimental set-up where one ham can be heated at a time.



The temperature measured in four different places during RF cooking of one ham. The figure shows that the speed of heating differs by up to approx. 10°C.



After RF cooking, the temperature was measured in several places to locate cold spots. The figure shows that the temperature reached varied between 50°C and 85°C. The coldest spots were in the outer ring of the ham.

Therefore, this RF cooking process was combined with heating in a water bath at 74°C for 10 minutes to cook the outside of the ham.

Challenge test documenting heat inactivation

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. are not relevant in this context as these are not inactivated until 90°C or more is reached.

The following strains were used for the experiment (Koch, A.G., 2017).

- *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*

The RF cooking did not inactivate all bacteria added to the cured ham. 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 on the selective substrate Slanetz at 45°C. Among the survivors measured on BHI-agar (Appendix 1) other bacteria than enterococci might have been isolated, for example spores from *Bacillus* species.

Growth during storage at 5°C or 8°C for 2 months

To investigate if cold spots occurred in the RF cooked ham, 5 new slices were made from each ham. These slices were divided into 3 samples, 1 core sample and 2 outer samples.

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 were survivors or if they also had multiplied during storage). The cold spot was located in the outer part of the ham. These samples are shown in Appendix 2. 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 bacteria added except for one ham sample cooked at 360 kJ only.

The result (Appendix 3) indicates 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, these RF cooked samples also showed presence of *Brochotrix* and *E. faecalis* indicating that these organisms were also 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 organism OR if these findings are partly due to small DNA fragments from bacteria inactivated at the heating process. It must be noted that high numbers of the bacteria represented by the detected DNA-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 meat or ingredients used were detected.

Traditional cooking to 72°C

As a control, inoculated hams were also cooked in a traditional cooking cabinet to 72°C. In these hams, the number of survivors after heat treatment was less than 1 log cfu/g. After two 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. 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. The results showed that the few survivors from heating to 72°C initiating growth at 8°C were dominated by *Lb. sakei*. No bacteria from the traditional cooking initiated growth during storage at 5°C.

In conclusion, RF cooking with 360 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, surviving bacteria from RF cooked hams were detected in one spot. The survivors had increased to 4 log cfu/g and were dominated by *Lb. sakei.*

After 2 months of storage at 8°C, survivors from one RF cooked ham cooked at 380 kJ showed 3 log cfu/g in the cooking gel. The sequencing indicated that these organisms were dominated by *Lb. sakei*.

Future development for RF cooking. In future projects on improving the technology and implementation of RF cooking, the following aspects must also be addressed:

- Hygienic design of the equipment
- Solutions for running a continuous heating process
- CCP – measuring time/temperature to avoid cold spots

Conclusion

The RF cooking + heating in a water bath for 10 minutes did not result in total inactivation of the bacteria added. *Enterococcus* spp. and *Lactobacillus sakei* survived the heat treatment, and in a few samples growth initiated during 2 months of storage at 5°C or 8°C.

As these strains survive in a few spots, it is concluded that the process must be improved to provide uniform heat treatment in the entire ham.

However, more research is also needed to investigate how *Lb. sakei* can survive RF cooking and traditional heating to 72°C in a cooking cabinet. It might be speculated how these bacteria are capable of protection against heat perhaps like spore formers OR how they are introduced to the product after heating.

Heat resistance of bacteria used in the challenge test compared to *salmonella* and *listeria.*

Bacteria	D-value (minutes)
E. faecium	$D_{70}=0.32$[a)]
	$D_{70}=1.73$[a)]
	$D_{68}=17$[b)]
	$D_{65}=55$[b)]
E. faecalis	$D_{65}=0.8$[b)]
	$D_{60}=8$[b)]
Lactobacillus sakei	$D_{60}=0.33$[b)]
Listeria	$D_{70}=0.14$[a)]
Salmonella	$D_{70}=0.32$[a)]

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

References

Koch, A.G. (2016) Microbial heat resistance in meat with different fat content, salt and water activity. Literature survey. Project 2003894.

Koch, A.G. (2017) Microbial inactivation during heat treatment with traditional cooking and RF cooking. Report. Project 2003894.

Appendix 1

Microbial count (log cfu/g) in inoculated ham before and after RF cooking (Koch, A.G., 2017)

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 days	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, analysed 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 log cfu/g; 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

Appendix 2

Microbial count (log cfu/g) in Ham S110, RF cooked at 360 kJ + post pasteurized and then stored at 5°C for 2 months. Outer part and core analysed separately (n=1). (Koch, A.G., 2017)

	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 days	<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

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). (Koch, A.G., 2017)

	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 days	<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.

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 each slice) (Koch, A.G., 2017)

	360 kJ (ham S112, 5 slices)			380 kJ (ham S101+S103, 5 slices each)		
Substrate	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 days	<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) One sample; all other <2
b) Two samples; all other <2

Appendix 3

Number of bacteria (BHI, 37°C, log cfu/g) and result from 16S Sequencing. Ham before and after RF cooking at Fraunhofer to 72°C and post-pasteurized at 74°C. (Koch, A.G., 2017)

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.

Number of bacteria (BHI, 37°C, log cfu/g) and result 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. (Koch, A.G., 2017)

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)