REDUCTION OF *C. BOTULINUM* SPORES AT THE MEAT SURFACE USING C-BAND MICROWAVES

Annemarie Gunvig[1*], Alexander Bardenshtein[3], Jens Scheller Andersen[4] and Mari Ann Tørngren[2]

1. Food Safety, DMRI Danish Technological Institute, 2630 Taastrup, Denmark
2. Meat Technology, DMRI Danish Technological Institute, 2630 Taastrup, Denmark
3. Packaging & Logistics, Materials, Danish Technological Institute, 2630 Taastrup, Denmark
4. Slaughterhouse Technologies, DMRI Danish Technological Institute, 2630 Taastrup, Denmark
[*]Corresponding author's email: agg@dti.dk

Abstract – The shelf life of fresh vacuum-packed meat is less than 10 days at 3-8°C, due to the risk of growth of *C. botulinum*. FSA recommends a heat treatment of 90°C for 10 minutes to achieve a 6 log reduction of *C. botulinum* spores. The purpose of this study was to test if a surface treatment by C-band microwaves is capable of faster inactivation of *C. botulinum* spores than conventional heat treatment at 90°C for 10 minutes. Beef pieces were inoculated with 10^5 heat resistant *C. botulinum* spores/cm^2. The samples were treated in a microwave oven (5.8 GHz, 750 W) or in a water bath at 90, 95, 97 and 99°C, respectively. The D-values for microwave treatment were 0.64 minutes at 100% of available power and 0.94 minutes at reduced power. The D-values for the water bath treatment at 90, 95, 97 and 99°C were 3.99, 3.69, 2.22 and 1.67 minutes, respectively. The results show that the time to obtain a 6 log reduction of *C. botulinum* spores can be reduced 6 times when using microwaves. The shorter treatment time with microwaves is due to a faster heating of the thin water film between the packaging material and the meat surface.

Key Words – *C. botulinum* spores, decontamination, extended shelf life, microwaves, vacuum-packed meat

I. INTRODUCTION

The Food Standard Agency (FSA) [1] recommends that the shelf life of fresh vacuum-packed meat is less than 10 days at 3-8°C, due to the risk of growth of *C. botulinum*. Under anaerobic conditions, *C. botulinum* can produce a very harmful toxin that can cause a fatal form of food poisoning. To reduce the risk of *C. botulinum*, FSA [1] recommends a heat treatment of 90°C for 10 minutes or a time and temperature combination sufficient to achieve a 6 log reduction of *C. botulinum* spores.

In semi-processed meat, a shelf life of more than 10 days at 5°C is expected. On whole meat roasts, the bacteria flora only occur at the surface [2]. By using microwaves at a specific frequency, only the surface of the meat is heated, resulting in a preservation of a red and juicy centre and an inactivation of the spores at the surface. The aim of this experiment was to test if a surface treatment with microwaves was able to inactivate *C. botulinum* spores faster than a heat treatment in water bath at 90°C for 10 minutes.

II. MATERIALS AND METHODS

Strains and preparation of spores: For safety reasons, four non-toxigenic and gas-producing strains of psychrotrophic *C. botulinum* were used in the study. Three strains (DB2, SPL242 and M114) were isolated from blown vacuum-packed meat as described by [3], and one strain (C 60E) was isolated in the study as described by [4]. 16S rDNA analysis of the non-toxigenic strains showed 99.7-99.9% homology to *C. botulinum* types B, E or F, and all strains were toxin-negative in the mouse assay.

The spores were cultivated in TPGY (Tryptone Pepton Glucose Yeast) and CMM (Cooked Meat Medium) and harvested as described in [5]. The inoculation cocktail was a mixture of all four strains.

Meat pieces: Bovine top sides without membranes were cut into cubes (4.5×4.5×4.5 cm) of approximately 100 g ± 10 g. The meat pieces were dried in a LAF bench for 15 minutes, and 1 ml of a spore solution (10^7 cfu/ml) was added to the surface, corresponding to about 10^5 spores per cm^2 of the meat. The meat pieces were vacuum-packed in airtight plastic bags (PETP 12/PEP LDPE 75).

Microwave treatment: In two experiments, samples were treated by microwaves from a magnetron (M 5801 J, Muegge; 5800 MHz, 750 W) built onto a cavity (MH0750S-812BA).

In the experiment #1, four times three samples (initial surface temperature 21°C) were treated at full power for 1, 2, 3 or 4 minutes. In the experiment #2, the initial surface temperature was increased to 45°C in a water bath at 55°C for 5 minutes before treatment in the

microwave oven. Immediately after, one bag at a time was treated at full power for 1 minute and thereafter at 25% of full power for 1, 2 or 2.5 minutes. The treatment was repeated five times for each treatment time.

In both experiments, the samples were immersed into ice water immediately after the treatment, and samples at time 0 minutes were untreated samples.

Water bath treatments: Parallel treatments were conducted in water bath at 90°C for 0, 2, 4 or 7 minutes; at 95°C for 0, 0.5, 1 and 2 minutes; at 97°C for 0, 0.5, 1 and 1.5 minutes and at 99°C for 0, 0.5, 1, 2 and 4 minutes. At each sampling time, three samples were analysed (five at 99°C)

Analysis of the treated meat pieces: Numbers of spores and vegetative cells of *C. botulinum* were analysed on SFP-agar and incubated for 3 days at 30°C. Samples for the spore analysis were heat treated at 75°C for 20 minutes before analysis on SFP-agar.

The D-value and regression coefficient (R^2) for each treatment was determined by linear regression from the plot of the logarithm for spore and vegetative counts against time.

III. RESULTS AND DISCUSSION

In Table 1, it is shown that the D-values for the water bath treatment decrease with increasing temperature as expected. The $D_{90°C}$ value of 3.99 minutes is in good agreement with the $D_{90°C}$ value of 4.4 minutes, determined for the strains in a laboratory meat system with momentary heating [6]. The low correlation coefficient of linear regression for $D_{95°C}$ is due to short treatment times.

At 90°C, heat treatment of 23.94 minutes results in a 6 log reduction of *C. botulinum* spores, which is much longer time than the FSA recommendation of 10 minutes. This could be caused by a higher heat resistance of the applied spores. The mean $D_{90°C}$ value for non-proteolytic and psychrotrophic *C. botulinum* spores (n = 175) is 1.39 minutes [7], equalling 8.4 minutes' heat treatment at 90°C to obtain a 6 log reduction. Some mesophilic *C. botulinum* strains can survive a heat treatment at 90°C for 10 minutes, because they are generally more heat resistant than the psychrotrophic *C. botulinum*. To avoid growth of the mesophilic *C. botulinum*, the storage temperature must be below 10°C.

Table 1. D-values for *C. botulinum* spores treated in microwave oven at 5.8 GHz and water bath at four different temperatures.

Exp. No	Equipment	Temperature (°C)	D-value (minutes)	R^2 reg.	Time to 6 log red. (minutes)
1	Water bath	90	3.99	0.816	23.94
1	Water bath	95	3.64	0.330	21.84
1	Water bath	97	2.22	0.588	13.32
2	Water bath	99	1.67	0.625	10.02
1	Microwawe[1]		0.68	0.864	4.08
2	Microwawe[2]		0.94	0.912	5.64

In experiment #1, the D-value for the microwave treatment was substantially lower than in experiment #2, which could be explained by the difference in effect of the microwave oven. Experiment #1 was conducted with 100% effect during the treatment of three samples, while during experiment #2 the effect was reduced to 25% after 1 minute of treatment of one sample. The effect was reduced to avoid leakers because of steam formation, which was observed after 1 minute of treatment. Steam formation indicates that the temperature was 100°C at the surface. In experiment #1, no inactivation was measured during the first 2 minutes of the treatment, which can be the result of a longer time to heat three samples instead of one sample, as applied in experiment #2. This indicates that the treatment time to obtain a specific inactivation increases with the amount of meat to be treated.

Comparison of D-values for *C. botulinum* spores determined by heating in water bath and microwave oven shows that spores are inactivated more effectively by the microwave treatment.

After treatment with microwaves at full power for 4 minutes or for 5.6 minutes at reduced power, a 6 log reduction of *C. botulinum* spores can be achieved. For the tested spores, a treatment time of 23.94 minutes at 90°C is necessary to obtain a 6 log reduction. This indicates that a treatment time, which is the 4-6 times shorter, is needed to achieve the same reduction when using microwave treatment.

In Table 2, it is shown that the D-values for vegetative cells heat-treated in water bath at 90-99°C are from 3.36 to 1.30 minutes, respectively. As expected, the D-values are lower than for the spores treated under the same conditions.

In the experiment #1, the D-value for the vegetative cells, treated with microwaves, was higher than for the spores, which could be due to large deviation between the counts of vegetative cells. While in the experiment

#2, the D-values for vegetative cells were less than for the spores, as expected.

Table 2. D-values for *C. botulinum* vegetative cells treated in microwave oven at 5.8 GHz and water bath at four different temperatures.

Exp. No	Equipment	Temperature (°C)	D-value (minutes)	R^2 reg.	Time to 6 log red. (minutes)
1	Water bath	90	3.36	0.815	20.16
1	Water bath	95	2.5	0.790	15.00
1	Water bath	97	1.66	0.787	9.96
2	Water bath	99	1.30	0.796	7.80
1	Microwawe[1]	[redacted]	1.01	0.622	6.06
2	Microwawe[2]	[redacted]	0.72	0.931	4.32

Even though the temperature of the meat surface achieved by immersion of packaged meat in hot water is approximately the same as the temperature achieved in the microwave heating process, the decontamination of the meat surface is faster when using microwaves. This is because the heating rate is faster for microwave heating than for the water bath heating. In water bath heating, a thin water film between the packaging film and the meat surface slows down the heat transfer process. As a result, the time necessary to obtain a 6 log reduction in 90°C water bath needs to be longer than if the temperature above 90°C could be instantaneously established right at the meat surface as it can be done using microwave heating.

Indeed, in case of decontamination using 5.8 GHz microwaves, the heating process can be described as follows: At the initial stage, the microwave energy is absorbed in the liquid film between the meat and the packaging film. Maximum 30-40% of the available microwave power reaches the meat surface since the power penetration depth of 5.8 GHz microwaves in the water is approximately 3.3 mm. This stage ends when the liquid film eventually begins to boil, and the temperature at the surface of the meat reaches 100°C. In its turn, the boiling process results in water steam generation and the consequent inflation of the packaging plastic bag. The increased headspace of the packaging is therefore filled with the water steam. This stage is essential for improvement of the decontamination results since the steam condensation with the release of enormous amount of heat happens right at the meat surface. Indeed, the surface is being continuously cooled since the heat percolates the meat product due to its relatively high thermal conductivity. This cooling promotes steam condensation on the meat surface. When the liquid film evaporates, the meat surface gets open for direct absorption of microwave energy since microwaves do not experience any essential energy loss in the moist air as they do in the water film.

In conclusion, the steam formation makes it easier for microwaves to heat the meat surface since the penetration depth of microwaves in steam is more than 10 times higher than it is in water.

The advantage of the microwave method is that the sterile red coloured centre is preserved during the fast decontamination of the surface on whole muscles (see Figure 1), meaning that a subsequent heat treatment at temperatures in the range from 55-63°C can be applied without affecting the food safety.

95°C, 21 minutes, 50 sec. | 99°C, 10 minutes | Microwaves 8.5 GHz, 3 minutes

Figure 1. Appearance of beef heat-treated at 95°C for 22 minutes, 99°C for 10 minutes and with microwaves (5.8 GHz) for 3 minutes. All treatments are equivalent to a 6 log reduction of *C. botulinum* spores.

On the other hand, in a view of its industrial implementation, the disadvantage of the microwave treatment is that larger amounts of meat require higher microwave power, which may not always be available.

IV. CONCLUSION

A 6 log reduction of the heat resistant *C. botulinum* spores can be achieved after 4 minutes of treatment with microwaves (5.8 GHz) with 100% effect. At reduced effect and preheating of the meat to 45°C, 5.6 minutes are required to achieve a reduction of 6 log. In water bath at 90°C, 95°C, 97°C and 99°C, 24, 22, 13.5 and 10 minutes are required to gain a reduction of 6 log.

The advantage of using microwaves is that the thin water film between the packaging material and the meat surface is immediately heated to 100°C. This results in an increase of the death rate of the spores.

The recommended 6 log reduction of *C. botulinum* spores can be achieved by surface decontamination of whole roasts of meat with microwave treatment. This indicates that it is possible to extend the shelf life of fresh vacuum-packed meat with a red centre to more than 10 days at 5°C.

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