Fiskeriforskning

Report 15/2000 • Published September 2000

Wood in Food

Hygienic limits and cleaning procedures. Partial report 2

Senior Scientist Grete Lorentzen, Fiskeriforskning and Consulting Engineer Ida Weider, Norwegian Institute of Wood Technology

Norut Group Ltd. consists of six research institutes located in Tromsø, Narvik and Alta. The Norut Group has 220 employees whose applied research and development encompasses a wide variety of interdisciplinary fields. Each subsidiary institute has a specific research emphasis, but common to all is activity centered around the polar and Barents regions.

Norut Group LTD consist of:

Fiskeriforskning (Norwegian Institute of Fisheries and Aquaculture Research), Tromsø and Bergen

Norut Information Technology Ltd, Tromsø

Norut Social Science Research Ltd, Tromsø

Norut Technology Ltd, Narvik

Norut Medicine and Health Ltd, Tromsø

Norut NIBR Finnmark AS, Alta

Fiskeriforskning (Norwegian Institute of Fisheries and Aquaculture Research) conducts research and development for the fisheries and aquaculture industry. The Institute covers virtually all links in the value chain – "from sea bottom to tabletop". Fiskeriforskning is a national research institute – owned by the Norut Group Ltd. (51 %) and the Norwegian Ministry of Fisheries (49 %). Located in Tromsø (head office) and Bergen, the facilities at Fiskeriforskning are an important part of the national infrastructure for fisheries and aquaculture research.

Fiskeriforskning have five main areas of research:
Seafood and industrial processing
Marine biotechnology and fish health
Aquaculture
Aquafeed and marine processing
Economics and marketing

Tromsø (head office)
Muninbakken 9-13, Breivika
P.O.B 6122
NO-9291 Tromsø
Norway
Tel.: +47 77 62 90 00
Fax: +47 77 62 91 00
E-mail:post@fiskeriforskning.no

Bergen
Kjerreidviken 16
NO-5141 Fyllingsdalen
Norway
Tel.: +47 55 50 12 00
Fax: +47 55 50 12 99
E-mail: office@fiskeriforskning.no

Internet: www.fiskeriforskning.no

Norwegian Institute of Fishery and Aquaculture Ltd.
N-9291 Tromsø
Phone +47 77 62 90 00, Telefax +47 77 62 91 00
E-mail: fiskforsk@norut.no
http://www.fiskforsk.norut.no/

REPORT

Accessibility:	*Report no:*	*ISBN-no:*
Open	15/2000	82-7251-454-0

Title: **Wood in Food – hygienic limits and cleaning procedures**	*Date:* 27 September 2000 *Number of pages and appendixes:* 35
Author(s): : Grete Lorentzen, Norwegian Institute of Fisheries and Aquaculture Ltd. and Ida Weider**,** Norwegian Institute of Wood Technology	*Director of Research:* Knut Sunnanå
Centre: Centre of Industrial Processing	*Project no.:* 8495
By agreement with: Norwegian Institute of Wood Technology	*Employers ref.:*
Head words: Wood, microorganisms, hygiene, heat treatment	

Summary:

This work is a part of the project "Wood in the Food industry", which have participants from all Nordic countries. The project is co-ordinated by the Norwegian Institute of Wood Technology and funded by the Nordic Wood. The aim of the present studies has been to study heat treatment of wood as a method to reduce the number of microorganisms to an acceptable hygienic level. The experiments are referring to activity no 4 and no 6 in the project; cleaning methods and hygienic limit values. Surfaces of wood were contaminated with either *Halobacterium salinarum* or microorganisms isolated from cod. The contaminated wood was then incubated at different time intervals and then heated at different temperatures and time intervals. The recovery of microbes after heat treatment was measured using both the swabbing and the contact method. The experiments show that heating can be used as a method to reduce the number of microorganisms on a wooden surface to an acceptable level. The required heating conditions (temperature, time) differ depending on the microorganisms present in the product and environment. Moist heat has better penetrating power than dry heat and causes a faster reduction of the number of living organisms at a given temperature. It seems that the time of incubation of the contaminated sample before the heat treatment is of vital importance to the detection of the microorganisms.

TABLE OF CONTENTS

1 INTRODUCTION 1

2 MATERIALS AND METHODS 3
- 2.1 Wooden samples 3
- 2.2 Density 3
- 2.3 Microorganisms 3
- 2.4 Media 4
- 2.5 Disinfection of samples 4
- 2.6 Contamination 4
- 2.7 Experimental structure 5
- 2.8 Measuring methods for recovery 5
- 2.9 Interpretation of the results 6

3 RESULTS AND DISCUSSION 7
- 3.1 Microorganisms isolated from cod, swabbing method 7
  - 3.1.1 Incubation of samples in 30 minutes 7
  - 3.1.2 Incubation of samples in 120 minutes 8
  - 3.1.3 Incubation of samples in 7 days 9
- *3.2 H.salinarum*, swabbing method 12
  - 3.2.1 Incubation of samples in 30 minutes 12
  - 3.2.2 Incubation of samples in 120 minutes 13
  - 3.2.3 Incubation of samples in 7 days 14
- 3.3 Microorganisms isolated from cod, contact method 14
  - 3.3.1 Incubation of samples in 30 minutes 14
  - 3.3.2 Incubation of samples in 120 minutes 16
  - 3.3.3 Incubation of samples in 7 days 17
- *3.4 H.salinarum*, contact method 18
  - 3.4.1 Incubation of samples in 30 minutes 18
  - 3.4.2 Incubation of samples in 120 minutes 19
  - 3.4.3 Incubation of samples in 7 days 20
- 3.5 Experiment with salted fish 20

4 CONCLUSION 23
- 4.1 Main conclusion 23
- 4.2 Methods for recovery 23
  - 4.2.1 Swabbing method 23
  - 4.2.2 Contact method 23
- 4.3 Experiments with salted fish 24

5 ACKNOWLEDGEMENTS 24

6 REFERENCES 25

7 APPENDIX 27

1 INTRODUCTION

Wood used to be the most common material for packaging, workbenches, shelves, tools, buildings, interiors etc., in the food industry in the Nordic countries. The use of wood has however decreased, and other materials like plastic, concrete, stainless steel and aluminium have taken its place. The reason for this negative development seems to be declining market demands, partly caused by legislation in Europe and elsewhere.

Despite this, nearly 1,5 million cubic meter of timber is used for pallets and packaging in the Nordic countries per year. These products are hence of great importance for the wood industry as the alternative production to packaging materials may be chips for pulp production. Based on this, a Nordic research project was initiated to find out more about the behaviour of wood in contact with foodstuff.

The main object of the project has been to collect data regarding wood products and their substitutes when used in the food industry, and to find suitable methods to identify and measure the growth of bacteria on wood and their substitutes.

This report is one in a series of reports where the results from the Nordic Wood 2 project no. P 98141 ”Wood in the Food Industry” are presented.

The aim of this work is to study heat treatment of wood as a method to reduce the number of microorganisms to an acceptable hygienic level. This work is referring to activity no 4 and no 6 in the project; cleaning methods and hygienic limit values. We have chosen heat treatment as a “cleaning method” in order to reduce or eliminate the growth and survival of microorganisms on a wooden surface. The hygienic limit values will be the minimum time and temperature condition that is required to obtain an acceptable hygienic level; that means an acceptable level of microbes. The definition of an acceptable hygienic level is based on the Norwegian guidelines for evaluation of hygienic results (Anon, 1992). When required, we have made additional definitions for acceptable hygienic limits.

In this experiment, we have contaminated surfaces of wood with either *Halobacterium salinarum* or microorganisms isolated from cod. The contaminated wooden samples were then incubated at different time intervals and then heated at different temperatures and time intervals. Based on the results from previous experiments (Lorentzen *et al*, 2000), we have used swabbing as a test method. In addition, we have used the contact method to confirm the results from the swabbing method.

We have also studied the risk of crosscontamination between the surface and the product by putting sterilised saltfish on wooden samples contaminated with *H. salinarum.* After 4 weeks of incubation, we studied the presence of *H.salinarum* in the salted fish.

The experiments are carried out at the Norwegian Institute of Fisheries and Aquaculture Ltd. located in Tromsø, while the Icelandic Fisheries Laboratories in Reykjavik and the Norwegian Institute of Wood Technology in Oslo have participated in planning the experiments.

The project is funded by the Nordic Industrial Fund through their program Nordic Wood 2 which is an R&D program for the Nordic wood industry. The Nordic timber and woodworking industry and national funding authorities in the Nordic countries have raised additional funding.

The project has a steering group with the following members:

- Heine Aven, chairperson Aven AS, Norway
- Marianne Moltke, deputy chair person Norwood AS, Denmark
- Stefan Nilsson Åsljunga Pallen AB, Sweden
- Bjarni Ingibergsson Limtré h.f., Iceland

Terje Apneseth, Norwegian Institute of Wood Technology (NTI), has been the Nordic project leader and editor of some of the part reports. From June 2000 Ida Weider, also from NTI, has taken over as project leader.

The following industries, organisations and research institutes have contributed with their know-how and services:

Denmark: Norwood A/S, Dansk Træemballage A/S, Dansk Teknologisk Institutt, Træteknik (DTI)

Iceland: SÍF. h.f., Limtré h.f., BYKO h.f., Samskip h.f., Vörubrétti h.f., Icelandic Fisheries Laboratory (IFL)

Norway: Aven AS, Høylandet treindustri AS, Saltfiskforum, Fiskeriforskning, Norsk Treteknisk Institutt (NTI)

Sweden: AB Gyllsjö Träindustri, Åsljunga Pallen AB, Strandbergs Trä och Pallindustri, Trätek, Institutet för träteknisk forskning,

The participants would like to forward their warm thanks to Nordic Industrial Fund and the national funding authorities in Denmark, Iceland, Norway and Sweden that have contributed to the funding of the project.

This partial report no 2 is written by: **Grete Lorentzen, Norwegian Institute of Fisheries and Aquaculture Ltd, Tromsø and Ida Weider, Norwegian Institute of Wood Technology.**

2 MATERIALS AND METHODS

2.1 Wooden samples

Spruce (*Picea abies*) was used as test specimen in the experiment. This soft wood, commonly used in production of pallets, was sampled from the pool of raw materials at Aven Treindustri A/S. Boards of approximately 19x100 mm, of good quality and length were chosen.

Figure 1 shows a schematic drawing how samples were obtained from the board. The boards numbered A-E were split in two along the centre. Samples approximating the size 50x50 mm were cut and marked as shown in the figure. Figure 2 shows the end surface of a wooden sample. The annual ring pattern shows the outside and pith side of the board. All markings were done on the outside, as the pallet wood manufacturer generally prefers that the pith-side of the board is up in the finished products. The reason is that when drying, the board will cup, tending for the annual rings to straighten. The pith-side of the board is normally being slightly convex as indicated in the figure. Only dry wooden samples have been used in the experiments.

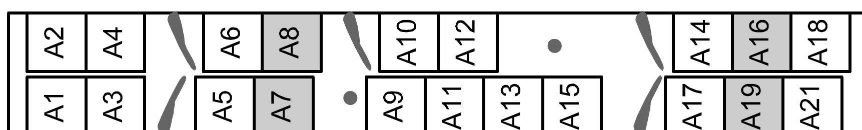


Figure 1. Schematic drawing of how samples were obtained from the board.

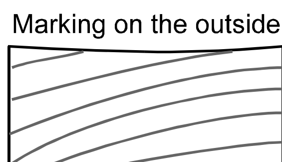


Figure 2. End surface of the wooden sample. Each sample was marked with a code on the outside.

2.2 Density

The density of wood may vary a lot within one board. According to the literature (Anon, 1999), the average density of spruce (*Picea abies*) is 430 kg/m^3, but due to the non-homogenous nature of wood, it may vary from 330 kg/m^3 to 680 kg/m^3.

2.3 Microorganisms

<u>*H. salinarum*</u>

This halophilic microorganism may be a problem in the salt-fish industry, and it is the most common cause of “pink” (pink spots) on salted fish.

When grown under optimum conditions, the *H. salinarum* may be rod or disc shaped. Some strains are highly pleomorphic even under optimum growth conditions. Most strains are strict aerobes, but facultative anaerobes growing with or without nitrate have been described in the literature (Larsen,1984). The optimum temperature is 40 °C, no growth occurs below 7-8 °C.

Most strains are able to survive up to 82 °C (van Klavern and Legendre, 1965). Colonies are pink, red, or red-orange, and are opaque to translucent and oxidase- and catalase- positive. Most isolates require at least 2.5 M (15 %) NaCl and 0,1 – 0,5 M Mg^{2+} for growth. Optimal growth conditions are 3,5 – 4,5 M (20 – 26 %) NaCl, and also grow well in saturated NaCl solution (>5 M, or > 29 % NaCl). The growth is relatively slow; generation times of 3-6 hrs are the fastest that have been reported in laboratory experiments (Larsen, 1984).

<u>Microorganisms isolated from cod</u>
To simulate the conditions in the fish industry in general, microorganisms isolated from cod have been used as a contaminant in the experiment. According to the literature (Hobbs G., 1991), 80 % of the total flora are covered by *Photobacterium*, *Pseudomonas, Alteromonas, Shewanella, Moraxella* and *Acinetobacter* strains.

2.4 Media

The microorganisms were detected by using specific media. To detect *H. salinarum*, a medium for halophilic microorganisms have been used. The method is described in appendix 1.

Prior to contamination of the wooden samples, the suspension containing *H. salinarum* was preincubated in 3-5 days in a liquid media (broth) under optimal growth conditions; 25 % NaCl, 37 °C, light, aerobic condition and continuous shaking.

Detection of microorganisms isolated from cod was performed using a standard plate count agar (PCA-Oxoid) supplemented with 1,5 % NaCl. The inoculum was preincubated in 3 days prior to contamination, and the cultures were incubated at room temperature (approximately 22°C).

2.5 Disinfection of samples

To avoid cross contamination, the wooden samples were disinfected in experiments involving microorganisms isolated from cod. In the experiments involving *H. salinarum*, disinfection of the wooden samples were not necessary since this microorganism demand extreme growth conditions; a high level of NaCl.

The samples of wood were disinfected in an autoclave at 121°C for 15 minutes. Before putting them into the autoclave, the samples were wrapped in aluminium paper, put in autoclaveable bags and sealed with an autoclaveable tape.

2.6 Contamination

A volume of 0.5 ml of the inoculum was spread evenly on the pith side of the wooden sample surface using a sterile pipette. The test organisms *H.salinarum* or microorganisms isolated from cod, will be referred as “contaminants” in the report.

2.7 Experimental structure

Table 1 shows the structure of the experiments. In experiment 1 and, 2, the swabbing method was used, while the contact method was used in experiment 3 and 4.

In experiment 5, sterilised salted fish was put on wooden surfaces contaminated with *H.salinarum*. This was done in order to study the risk of crosscontamination; transfer of *H.salinarum* from the wooden surface to the salted fish.

In order to simulate an extremely high contamination, the content of microbes in the contamination cultures was at least 10^8 CFU/ml in all experiments. Both moist and dry heat was used in all experiments.

Table 1. Structure of the experiments.

Experi-ment (no)	Test micro-organism	Method for recovery	Incubation temperature (C°)/ incubation timeprior to heat treatment (min)	Heating temperature (C°) / heating time (min)	Interpretation of the results	Results (chapter)
1	Microbes Isolated from cod	Swabbing	20 / 30, 120, 7 days	60, 75, 90, 100/30, 60, 90, 120, 240	Scale *) (1–6)	3.1
2	*H.salinarum*		37 / 30, 120, 7 days	60, 75, 90/30, 60, 90, 120	Scale *) (1–7)	3.2
3	Microbes isolated from cod	Contact	20 / 30, 120, 7 days	60, 75, 90/30, 120	Scale *) (1–5)	3.3
4	*H.salinarum*		37 / 30, 120, 7 days	60, 75, 90/30, 120	Scale *) (1–5)	3.4
5	*H.salinarum*	Analysis of saltfish	37 / 30, 120, 7 days	60, 75, 90/30, 120	CFU/g **)	3.5

*) See appendix 2
**) See appendix 1

2.8 Measuring methods for recovery

Based on results from previous experiments (Lorentzen *et al*, 2000), two methods for recovery of the microorganisms from the contaminated wooden samples were chosen; swabbing and contact method.

The recovered microbes were cultivated in petri dishes, and incubated at optimum growth conditions for the test organism. Samples with *H.salinarum* were incubated at 37°C, under light and aerobic condition, and samples with microorganisms isolated from cod were incubated at room temperature. The method for detection of halophilic microbes is described in appendix 1. All samples were performed in duplicate.

Swabbing

After adding the contaminant to the wooden sample, the surface was swabbed by using a sterile cotton wool (swab), which was predipped in sterile peptone / salt water solution. The samples were collected by stroking the surface according to a defined pattern. Then, the swab was stirred in the sterile peptone / salt water liquid. The numbers of microbes in the salt / water liquid was determined by plate counting.

Contact method

After contamination, the wood samples were put on a surface of nutrient agar in a petri dish for 2 minutes.

All petri dishes were put in a plastic bag in order to avoid drying of the samples.

2.9 Interpretation of the results

Swabbing method

To interpret the results from the swabbing method, a grading system developed by Statens næringsmiddeltilsyn, Norway (1992), was used. The grading system is shown in appendix 2. In the experiments with *H.salinarum*, the grading scale was extended to grade 7. In the salt fish industry, no *H.salinarum* is allowed to be present in the saltfish (Anon, 1997). Based on this, grade 1 (< 25 CFU), is not sufficient. We added one grade to the scale; <1 CFU, and named "grade 1".

Contact method

When interpreting the results for the contact method, counting the colonies was impossible due to the high concentration of contaminants. Instead, a grading system scaled from 1 – 5 based on the intensity of growth was used. Grade "1"is no visible growth, and grade "5" is too numerous for counting (appendix 2).

3 RESULTS AND DISCUSSION

Most of the results from the experiments are mainly presented as figures. In appendix 3, 4, 5 and 6, the same results are summarised and presented in tables.

3.1 Microorganisms isolated from cod, swabbing method

The figures 3 - 8 show the results from the swabbing method of wooden surfaces where microorganisms isolated from cod are used as a contaminant.

3.1.1 Incubation of samples in 30 minutes

Figure 3 shows results from experiment using 30 minutes incubation of wood samples, then heating at 60, 75 and 90°C (moist heat) in 30, 60, 90 and 120 minutes, while figure 4 shows the results from the corresponding experiment using dry heat.

Figure 3 and 4 show no reduction of CFU when heating at 60 and 75°C, except for 120 minutes at 75°C. However, at 90°C, there is a reduction of CFU according to the time of heating.

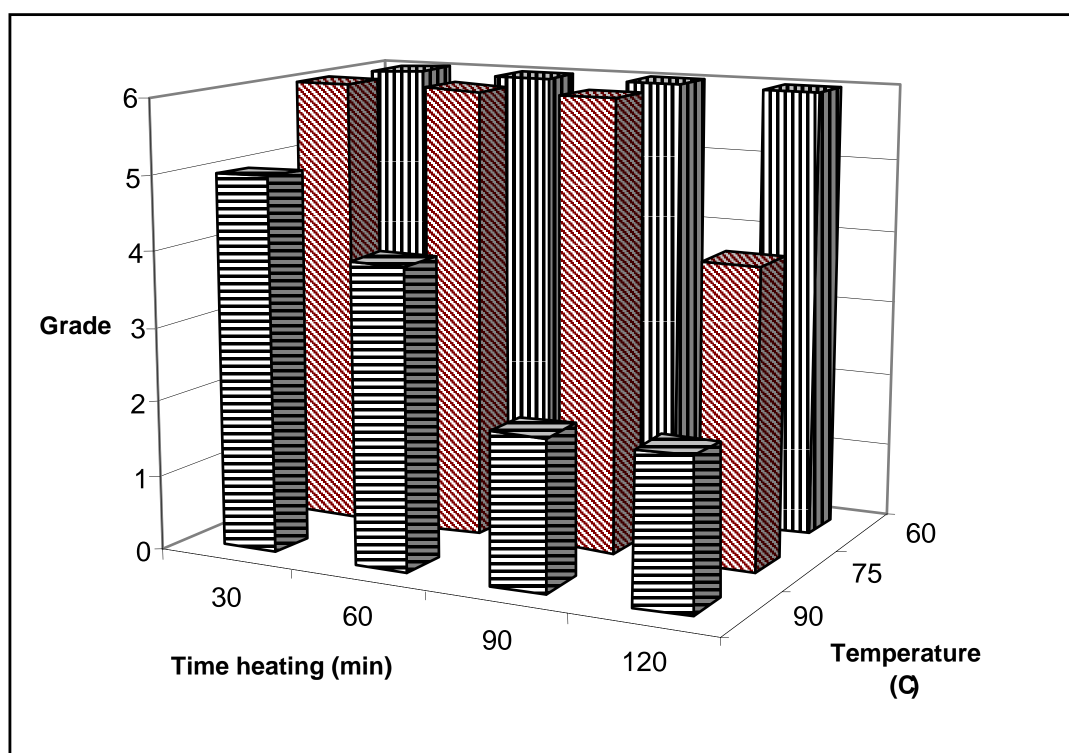


Figure 3. Wood samples with 0,5 ml microorganisms isolated from cod (10^8 CFU/ml) as a contaminant, incubated in 30 minutes at room temperature, and then heated at 60 (II) 75 (\\) and 90 (=) °C in 30, 60, 90 and 120 minutes. The samples were heated with moisture.

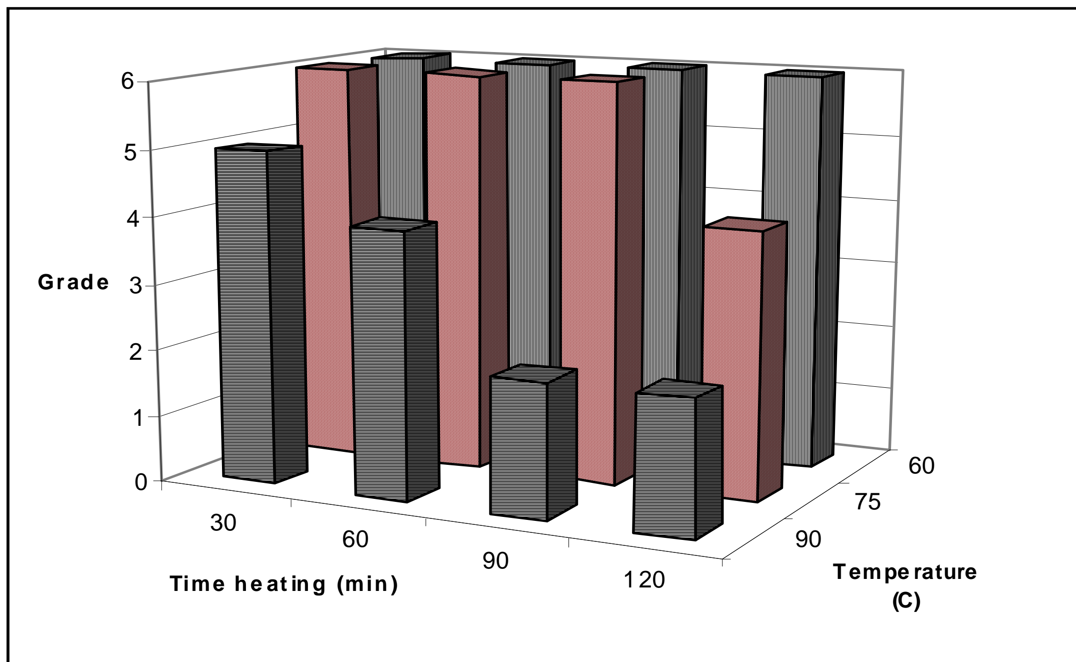


Figure 4. Wood samples with 0,5 ml microorganisms isolated from cod (10^8 CFU/ml) as a contaminant, incubated in 30 minutes at room temperature, and then heated at 60 (II) 75 (\\\) and 90 (=) °C in 30, 60, 90 and 120 minutes. The samples were heated in dry air.

3.1.2 Incubation of samples in 120 minutes

Figure 5 shows results from experiment using 120 minutes incubation of wood samples, prior to heating at 60, 75 and 90°C (moist heat) in 30, 60, 90 and 120 minutes, while figure 6 shows the corresponding experiment using dry heat. The results show that moist heat is more effective compared to dry heat in order to lower the CFU on the surface.

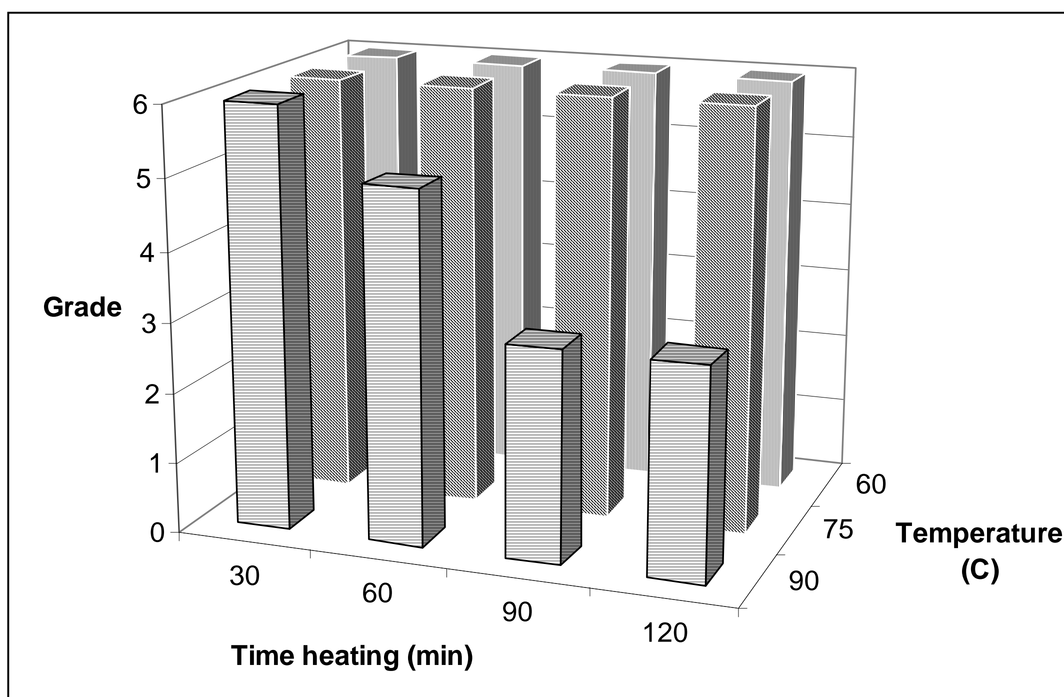


Figure 5. Wood samples with 0,5 ml microorganisms isolated from cod (10^8 CFU/ml) as a contaminant, incubated in 120 minutes at room temperature, and then heated at 60 (II) 75 (\\\) and 90 (=) °C in 30, 60, 90 and 120 minutes. The samples were heated with moisture.

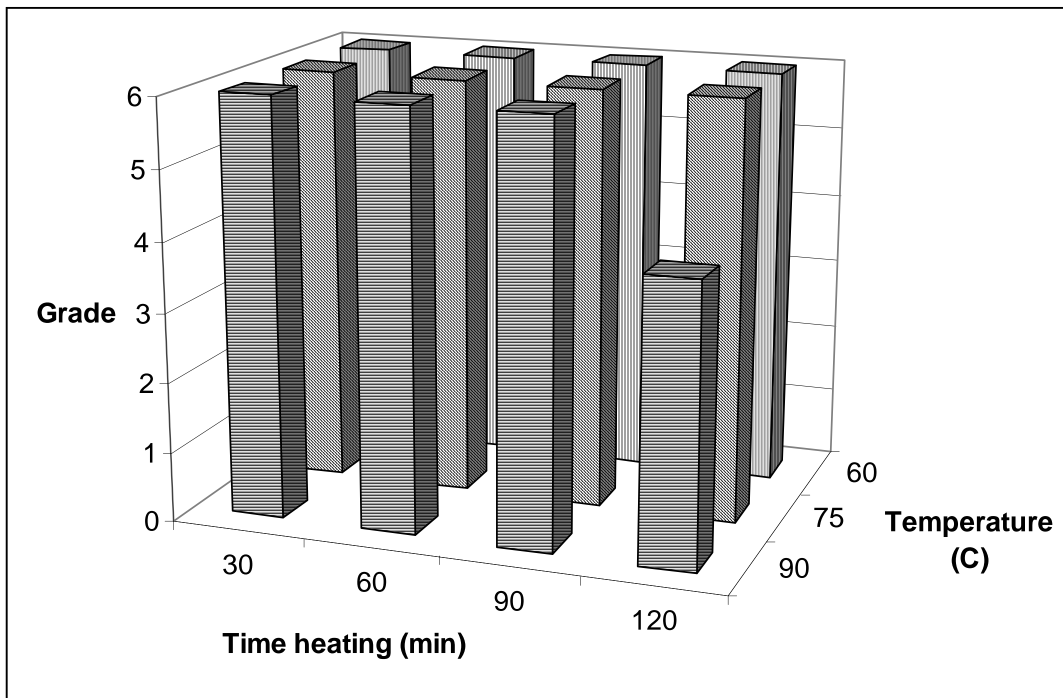


Figure 6. Wood samples with 0,5 ml microorganisms isolated from cod (10^8 CFU/ml) as a contaminant, incubated in 120 minutes at room temperature, and then heated at 60 (II) 75 (\\) and 90 (=) °C in 30, 60, 90 and 120 minutes. The samples were heated in dry air.

3.1.3 Incubation of samples in 7 days

Figure 7 shows results from experiment using 7 days incubation of wood samples, before heating at 60, 75 and 90°C (moist heat) in 30, 60, 90 and 120 minutes, while figure 8 show the corresponding experiment using dry heat. The results presented in figure 3 - 8 shows that grade 1 was not obtained by any of the heating conditions. A summary of these results is shown in appendix 3.

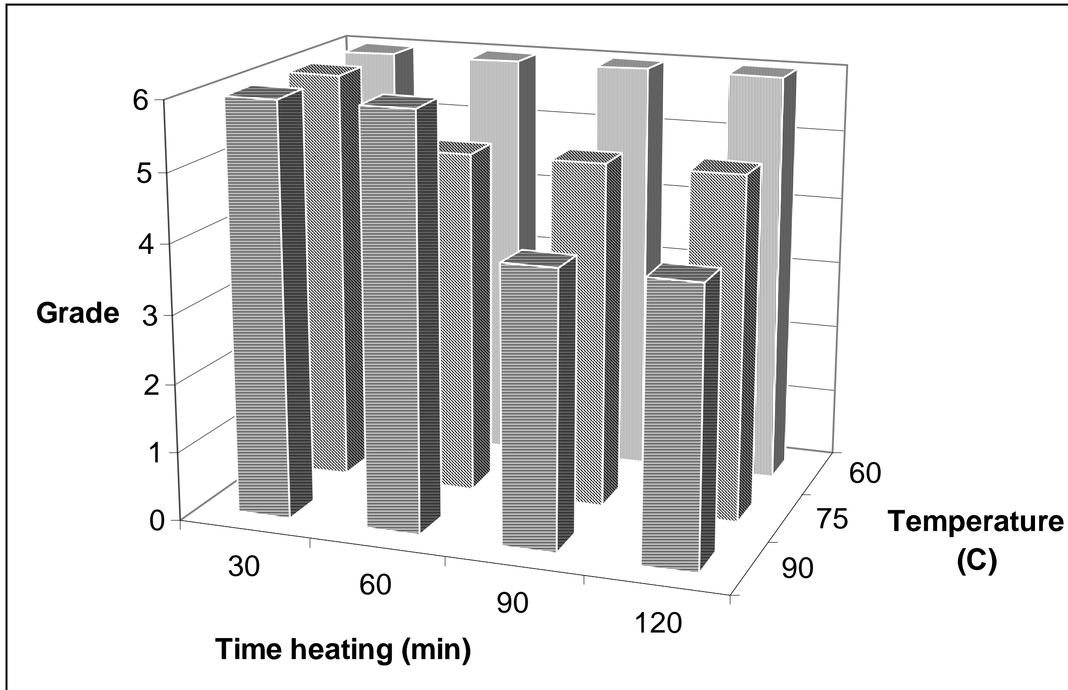


Figure 7. Wood samples with 0,5 ml microorganisms isolated from cod (10^8 CFU/ml) as a contaminant, incubated in 7 days at room temperature, and then heated at 60 (II) 75 (\\\) and 90 (=) °C in 30, 60, 90 and 120 minutes. The samples were heated with moisture.

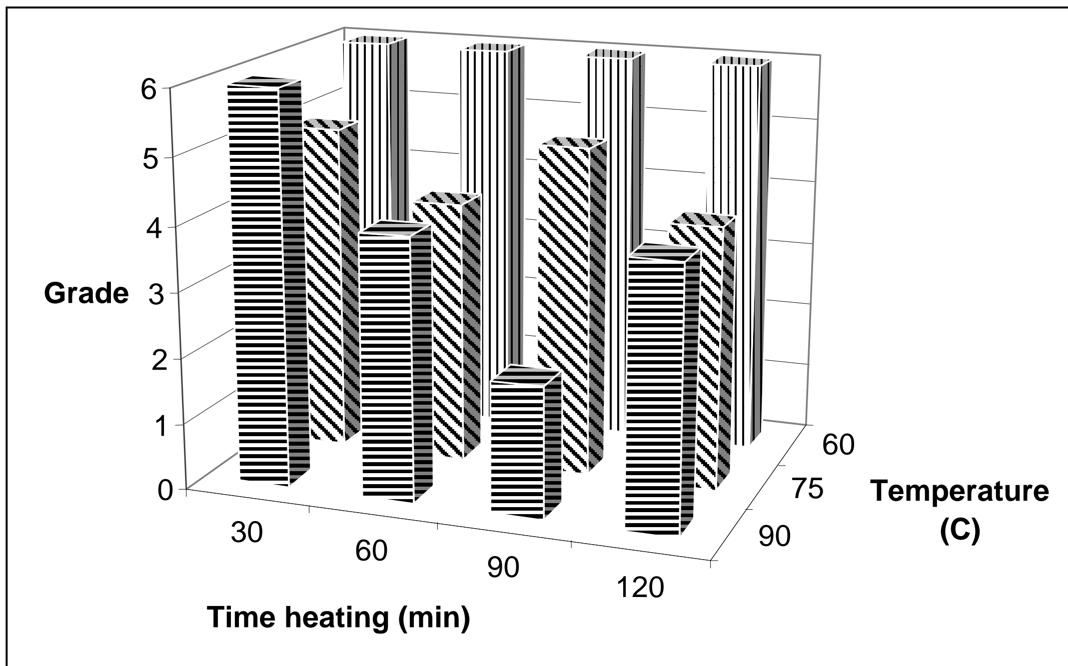


Figure 8. Wood samples with 0,5 ml microorganisms isolated from cod (10^8 CFU/ml) as a contaminant, incubated in 7 days at room temperature, and then heated at 60 (II) 75 (\\\) and 90 (=) °C in 30, 60, 90 and 120 minutes. The samples were heated in dry air.

In order to reduce the number of microorganisms after heating, the samples were heated in moist heat at 100°C up to 240 minutes. The results are shown in table no 2.

*Table 2. Detection of microorganisms isolated from cod on a wooden surface when heating at 100° C, moist heat (****** is equal to > 300 CFU/ml and * is equal to 1 – 25 CFU/ml). The microorganisms were analysed using the swabbing method.*

Time heating (min)	Time of incubation at room temperature before heating to 100°C moist heat		
	30 min	120 min	7 days
30	******	*****	****
60	*****	***	***
90	**	***	**
120	**	***	*
150	*	*	*
180	*	*	*
210	*	*	*
240	*	*	*

To obtain "*", heat treatment at 100°C in 150 minutes is sufficient for samples incubated in 30 and 120 minutes with contamination. An incubation of 7 days prior heat treatment, require 120 minutes at 100°C to obtain "*".

3.2 *H.salinarum*, swabbing method

3.2.1 Incubation of samples in 30 minutes

Figure 9 shows results from experiments with 30 minutes incubation of wood samples, then heating at 60, 75 and 90°C (moist heat) in 30, 60, 90 and 120 minutes, while figure 10 shows the results from the corresponding using dry heat. The figures 9 and 10 show a reduction of CFU when raising the temperature from 60 to 75°C. At 90°C almost all samples obtained grade 1.

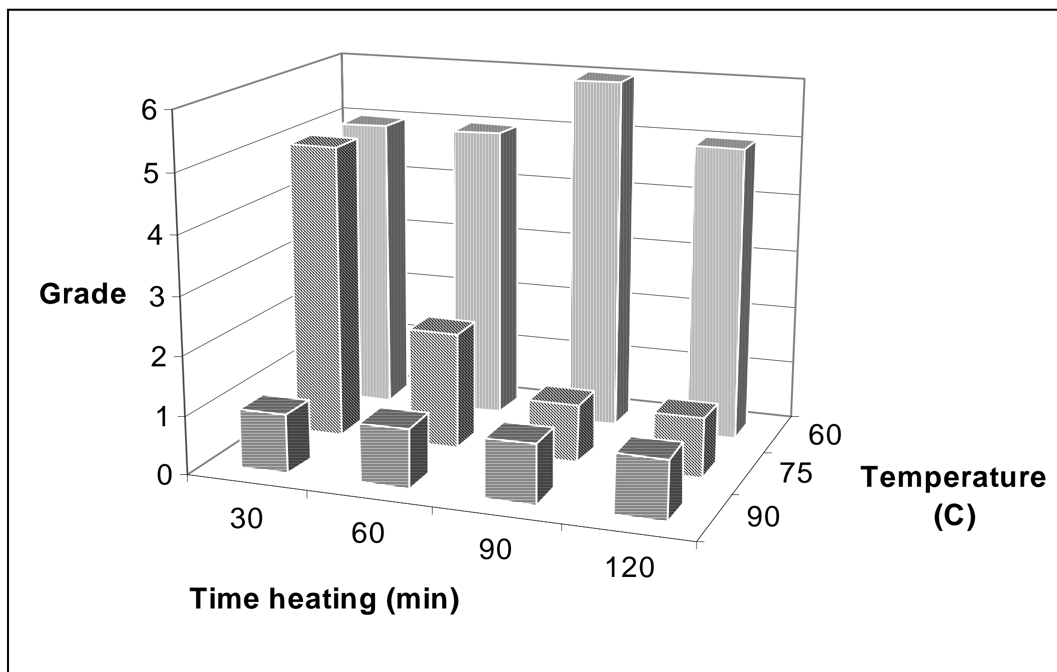


Figure 9. Wood samples with 0,5 ml H.salinarum (10^8 CFU/ml) as a contaminant, incubated in 30 minutes at room temperature, and then heated at 60 (II) 75 (\\) and 90 (=) °C in 30, 60, 90 and 120 minutes. The samples were heated with moisture.

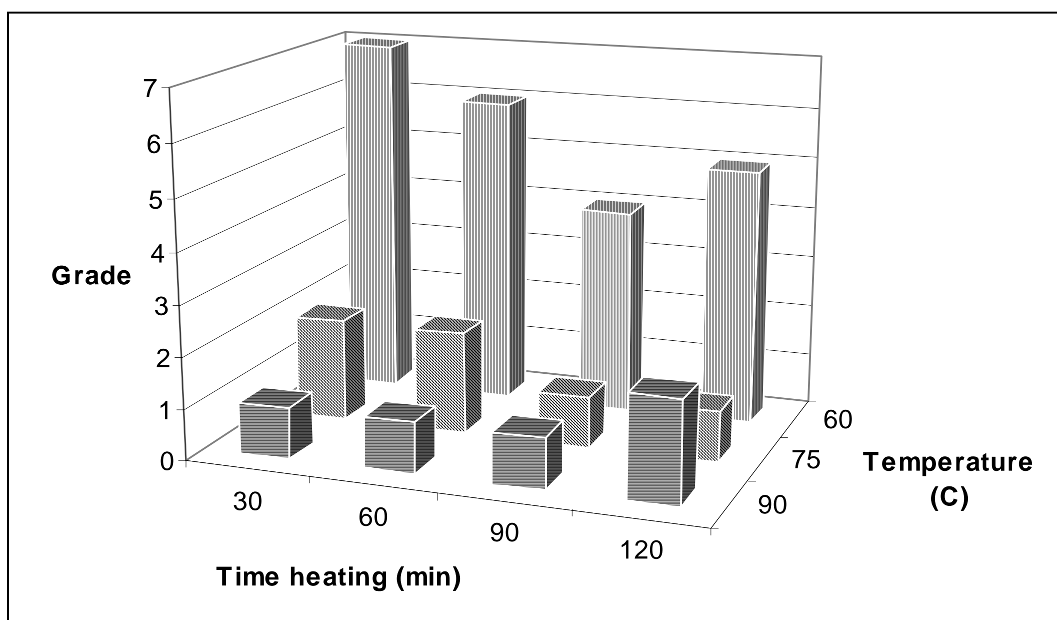


Figure 10. Wood samples with 0,5 ml H.salinarum (10^8 CFU/ml) as a contaminant, incubated in 30 minutes at room temperature, and then heated at 60 (II) 75 (\\) and 90 (=) °C in 30, 60, 90 and 120 minutes. The samples were heated in dry air.

3.2.2 Incubation of samples in 120 minutes

Figure 11 shows results from experiment with 120 minutes incubation of wood samples, then heating at 60, 75 and 90°C (moist heat) in 30, 60, 90 and 120 minutes, while figure 12 shows the results from the corresponding experiments using dry heat. The figures show that 75°C in 60 minutes is sufficient to obtain grade 1, when the time of incubation before heating is 120 minutes.

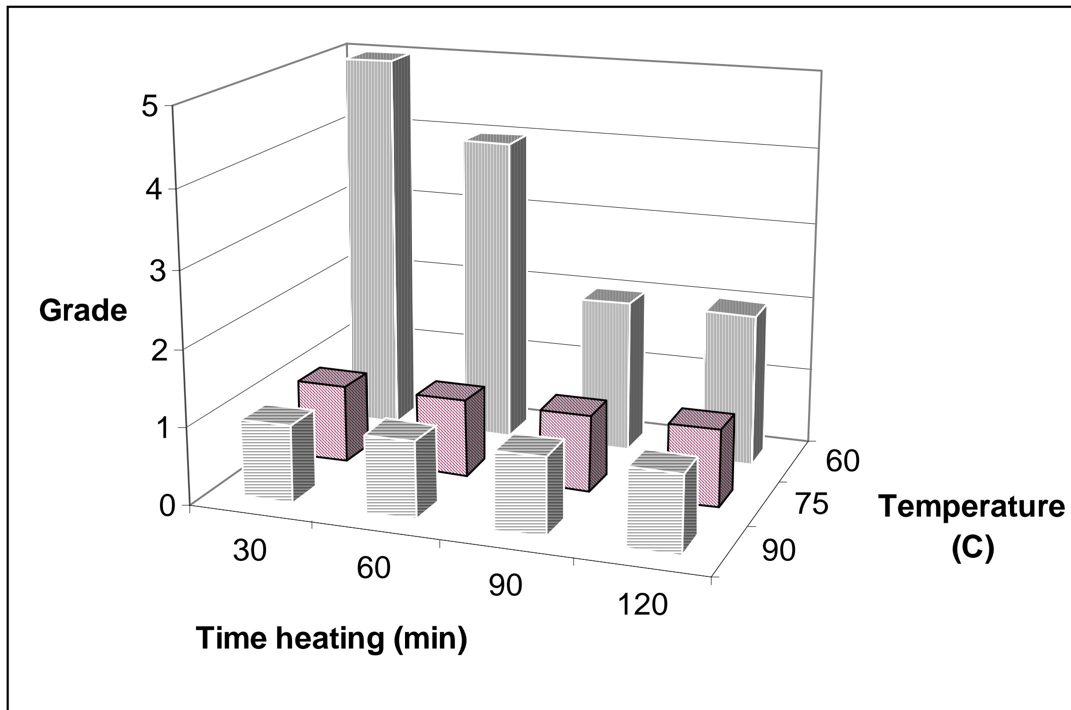


Figure 11. Wood samples with 0,5 ml H.salinarum (10^8 CFU/ml) as a contaminant, incubated in 120 minutes at room temperature, and then heated at 60 (II) 75 (\\) and 90 (=) °C in 30, 60, 90 and 120 minutes. The samples were heated with moisture.

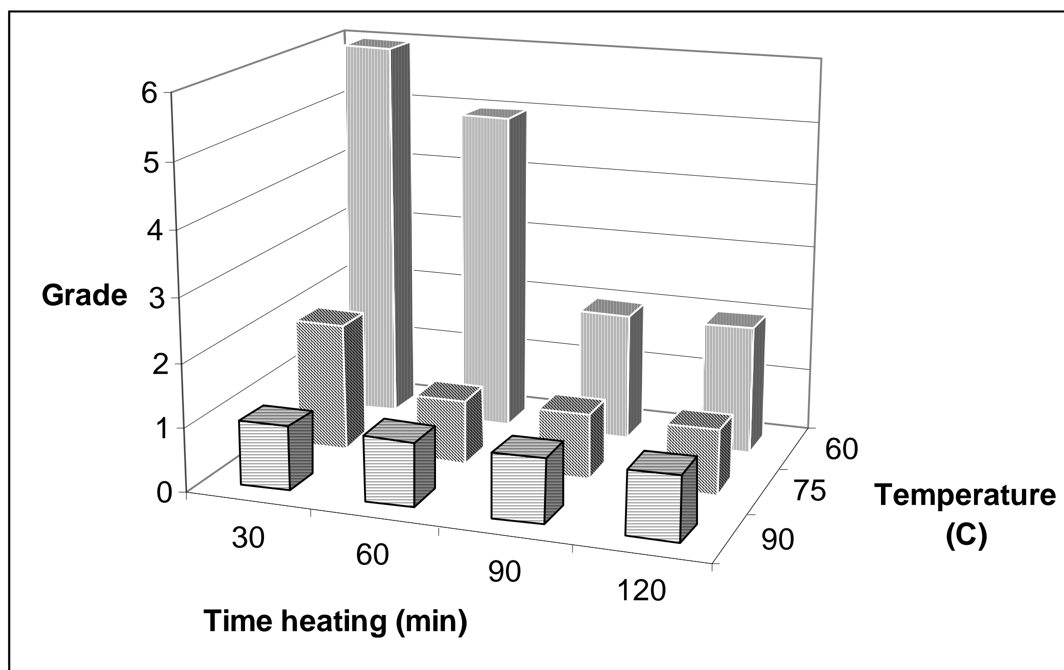


Figure 12. Wood samples with 0,5 ml H.salinarum (10^8 CFU/ml) as a contaminant, incubated in 120 minutes at room temperature, and then heated at 60 (II) 75 (\\) and 90 (=) °C in 30, 60, 90 and 120 minutes. The samples were heated in dry air.

3.2.3 Incubation of samples in 7 days

Figure 13 shows results from experiment with 7 days incubation of wood samples, then heating at 60, 75 and 90°C for 30, 60, 90 and 120 minutes. Due to similarity of the results from both moist and dry heat, the figure represents results from both series.

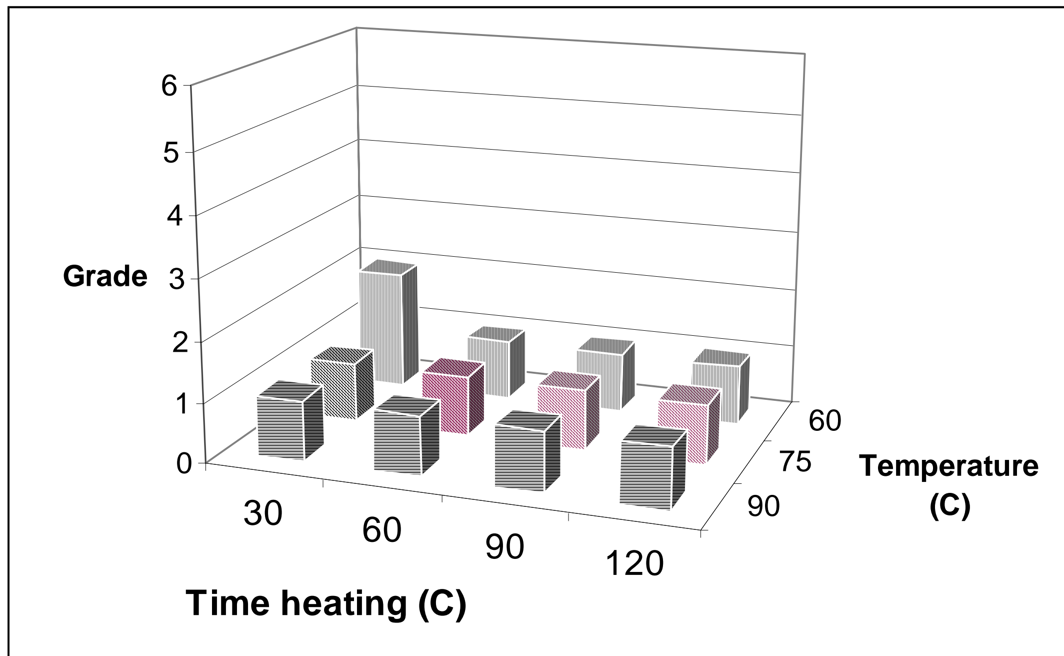


Figure 13. Wood samples with 0,5 ml H.salinarum (10^8 CFU/ml) as a contaminant, incubated in 7 days at room temperature, and then heated at 60 (II) 75 (\\) and 90 (=) °C (dry and moist heat) in 30, 60, 90 and 120 minutes

Figures 9 - 13 shows that heating at 75°C for 60 minutes is sufficient for samples incubated in 30 minutes with contamination. When the time of incubation was extended to 120 minutes, heating at 60°C in 90 minutes was sufficient to obtain "grade 1". When the samples were incubated for 7 days before heating, 60°C in 60 minutes was sufficient to obtain "grade 1". A summary of the results in figure 7-11 is shown in appendix 4.

3.3 Microorganisms isolated from cod, contact method

To evaluate the effect of the swabbing method, some corresponding samples for the contact method were made. The contact method is easy to perform, and convenient for screening the hygienic conditions of the wood (Lorentzen *et al*, 2000). In this experiment, microorganisms isolated from cod and *H.salinarum* were used as test organisms. Figure 14 – 19 show the results by using microorganisms isolated from cod and the figures 20 - 24 show the results using *H.salinarum* as a test organism.

3.3.1 Incubation of samples in 30 minutes

Figure 14 shows results from experiment using 30 minutes incubation of wood samples, prior to heating at 60, 75 and 90°C (moist heat) in 30 and 120 minutes, while figure 15 shows the results from the corresponding experiment using dry heat. The figures show a reduction of CFU when the heating time is 120 minutes.

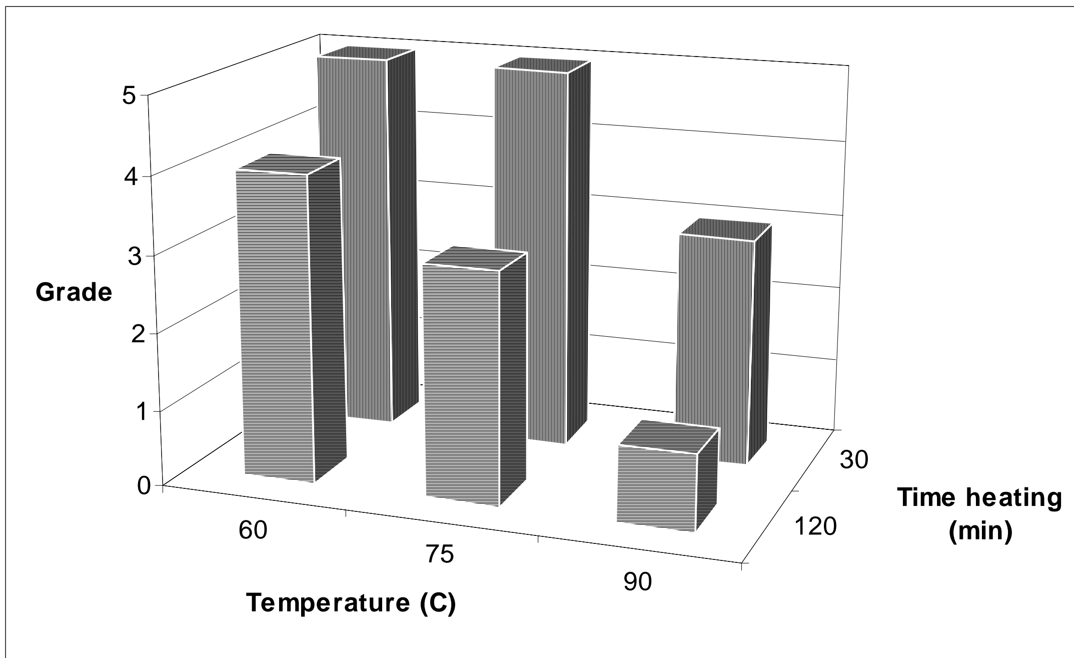


Figure 14. Wood samples with 0,5 ml microorganisms isolated from cod (10^8 CFU/ml) as a contaminant, incubated in 30 minutes at room temperature, and then heated at 60, 75 and 90 °C in 30 (II) and 120 (=) minutes. The samples were heated with moisture.

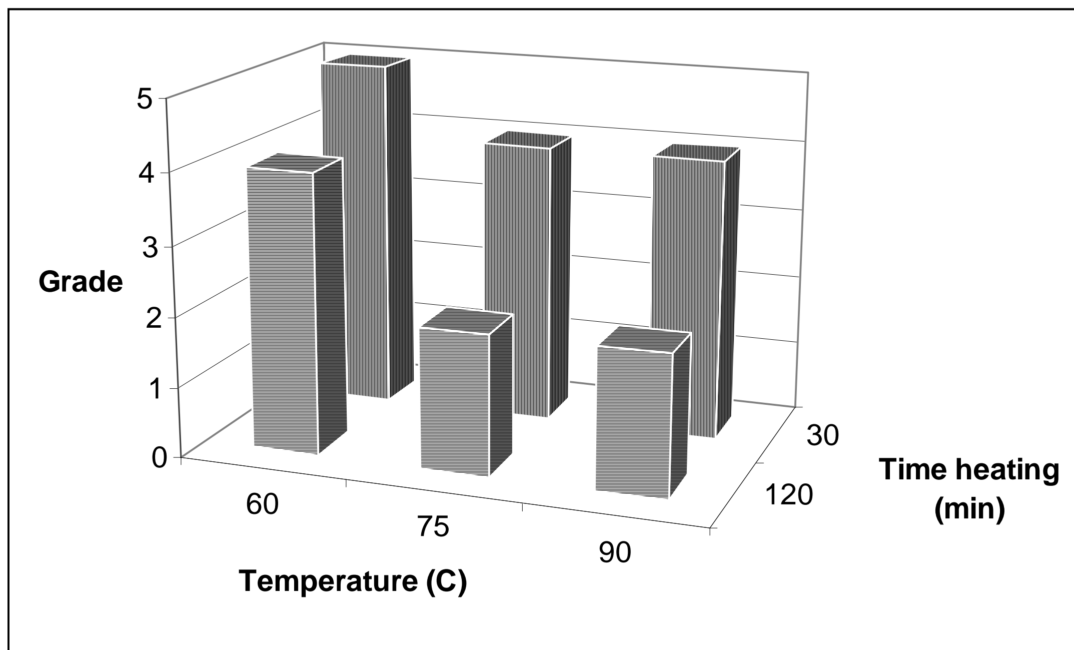


Figure 15. Wood samples with 0,5 ml microorganisms isolated from cod (10^8 CFU/ml) as a contaminant, incubated in 30 minutes at room temperature, and then heated at 60, 75 and 90 °C in 30 (II) and 120 (=) minutes. The samples were heated in dry heat.

3.3.2 Incubation of samples in 120 minutes

Figure 16 shows results from experiment with 120 minutes incubation of wood samples, then heating at 60, 75 and 90°C (moist heat) in 30 and 120 minutes, while figure 17 shows the results from the corresponding experiment using dry heat.

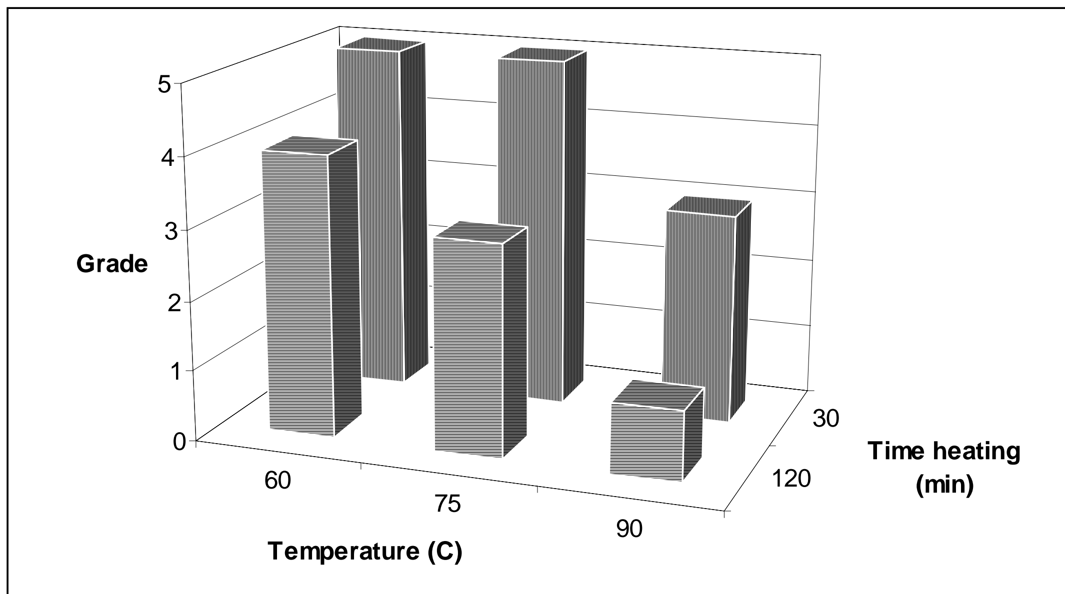


Figure 16. Wood samples with 0,5 ml microorganisms isolated from cod (10^8 CFU/ml) as a contaminant, incubated in 120 minutes at room temperature, and then heated at 60, 75 and 90°C in 30 (II) and 120 (=) minutes. The samples were heated with moist heat.

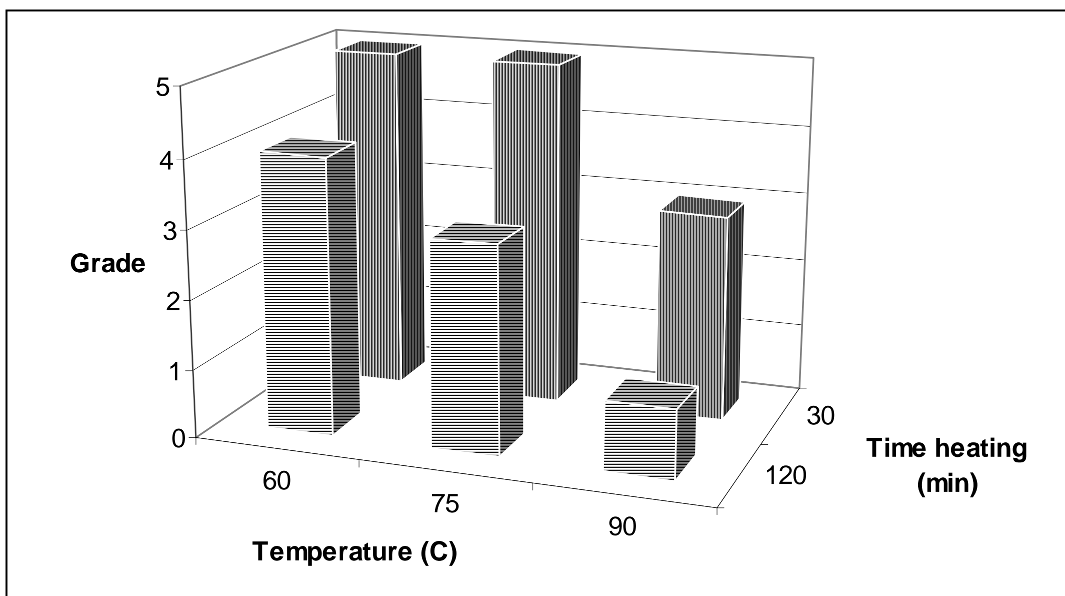


Figure 17. Wood samples with 0,5 ml microorganisms isolated from cod (10^8 CFU/ml) as a contaminant, incubated in 120 minutes at room temperature, and then heated at 60, 75 and 90°C in 30 (II) and 120 (=) minutes. The samples were heated in dry heat

3.3.3 Incubation of samples in 7 days

Figure 18 shows results from experiment with 7 days incubation of wood samples, then heating at 60, 75 and 90°C (moist heat) in 30 and 120 minutes, while figure 19 shows the results from the corresponding experiment using dry heat.

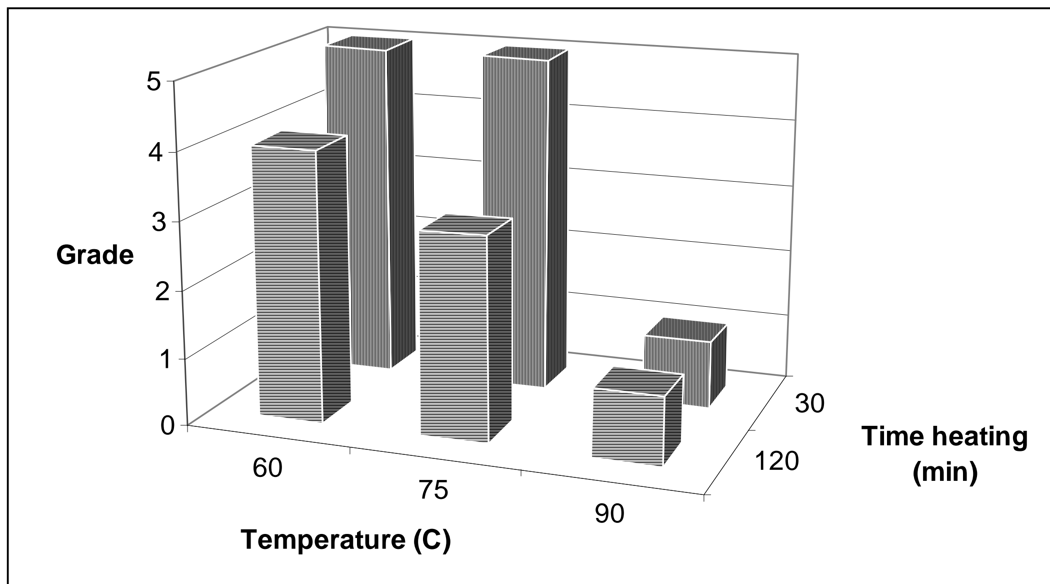


Figure 18. Wood samples with 0,5 ml microorganisms isolated from cod (10^8 CFU/ml) as a contaminant, incubated in 120 minutes at room temperature, and then heated at 60, 75 and 90°C in 30 (II) and 120 (=) minutes. The samples were heated in moist heat.

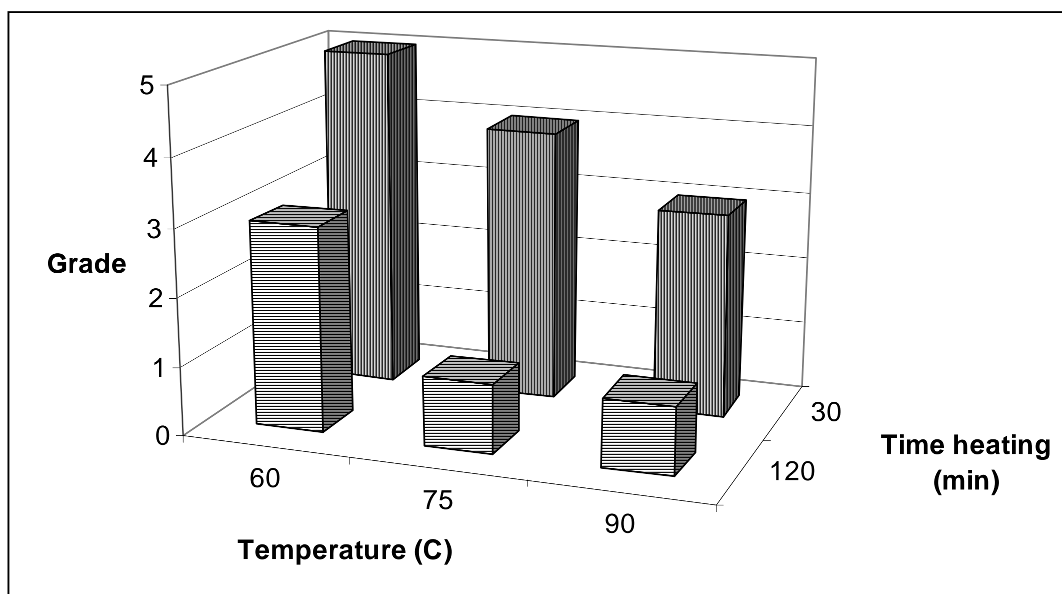


Figure 19. Wood samples with 0,5 ml microorganisms isolated from cod (10^8 CFU/ml) as a contaminant, incubated in 120 minutes at room temperature, and then heated at 60, 75 and 90°C in 30 (II) and 120 (=) minutes. The samples were heated in dry heat.

To obtain “grade 1”, the results presented in figures 14 - 19 show that heating at 90°C in 120 minutes is necessary for samples incubated with the contaminant in 120 minutes and 7 days. None of the applied heating conditions gave acceptable results (grade 1) for samples. incubated with the contaminants in 30 minutes before heating. A summary of the results from figures 14 - 19 is shown in appendix 5.

3.4 *H.salinarum*, contact method

3.4.1 Incubation of samples in 30 minutes

Figure 20 show results from experiment with 30 minutes incubation of wood samples, then heating at 60, 75 and 90°C (moist heat) in 30 and 120 minutes, while figure 21 shows the results from the corresponding experiment using dry heat. According to the figures, heating at 60°C with moist heat has a better killing effect compared to dry heat.

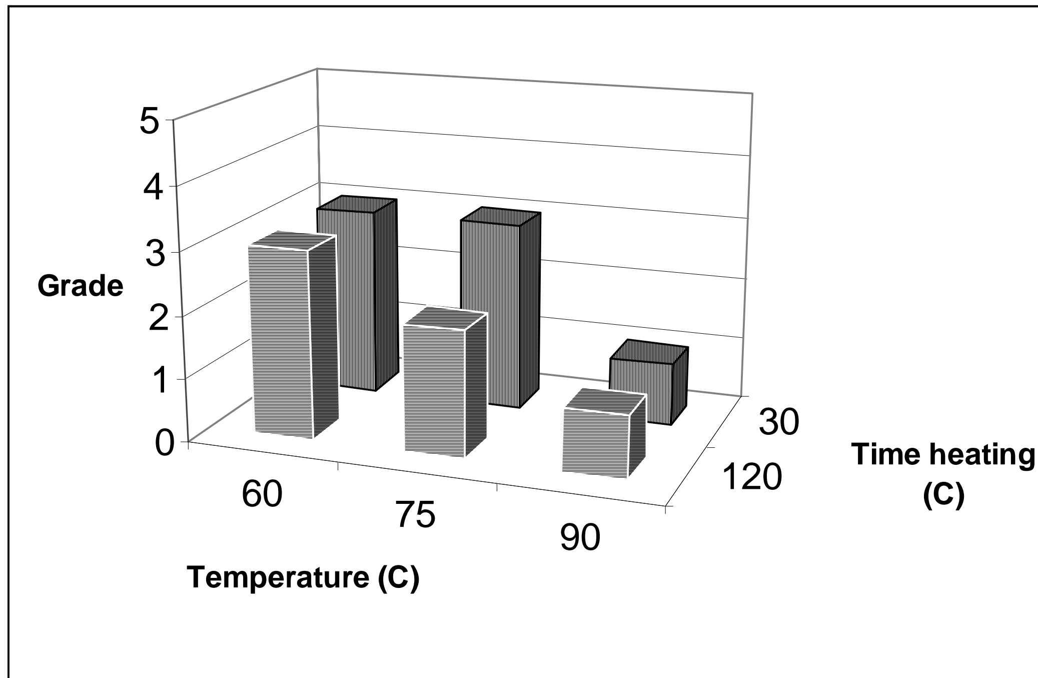


Figure 20. Wood samples with 0,5 ml H.salinarum (10^8 CFU/ml) are as a contaminant, incubated in 120 minutes at room temperature, and then heated at 60, 75 and 90°C in 30 (II) and 120 (=) minutes. The samples were heated with moist heat.

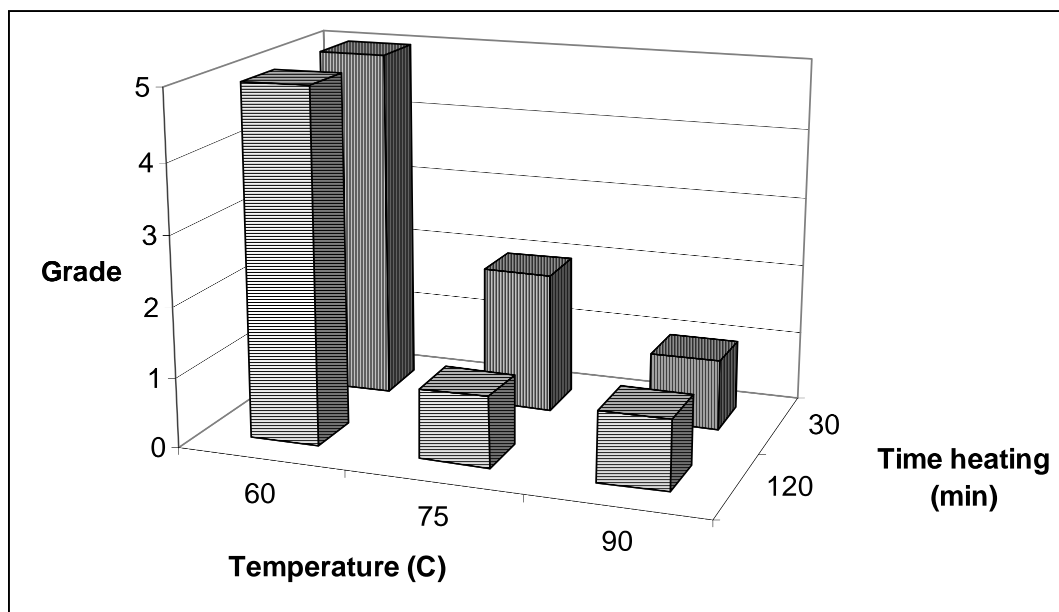


Figure 21. Wood samples with 0,5 ml H.salinarum (10^8 CFU/ml) as a contaminant, incubated in 120 minutes at room temperature, and then heated at 60, 75 and 90°C in 30 (II) and 120 (=) minutes. The samples were heated in dry heat.

3.4.2 Incubation of samples in 120 minutes

Figure 22 shows results from experiment with 120 minutes incubation of wood samples, then heating at 60, 75 and 90°C (moist heat) in 30 and 120 minutes, while figure 23 shows the results from the corresponding experiment using dry heat.

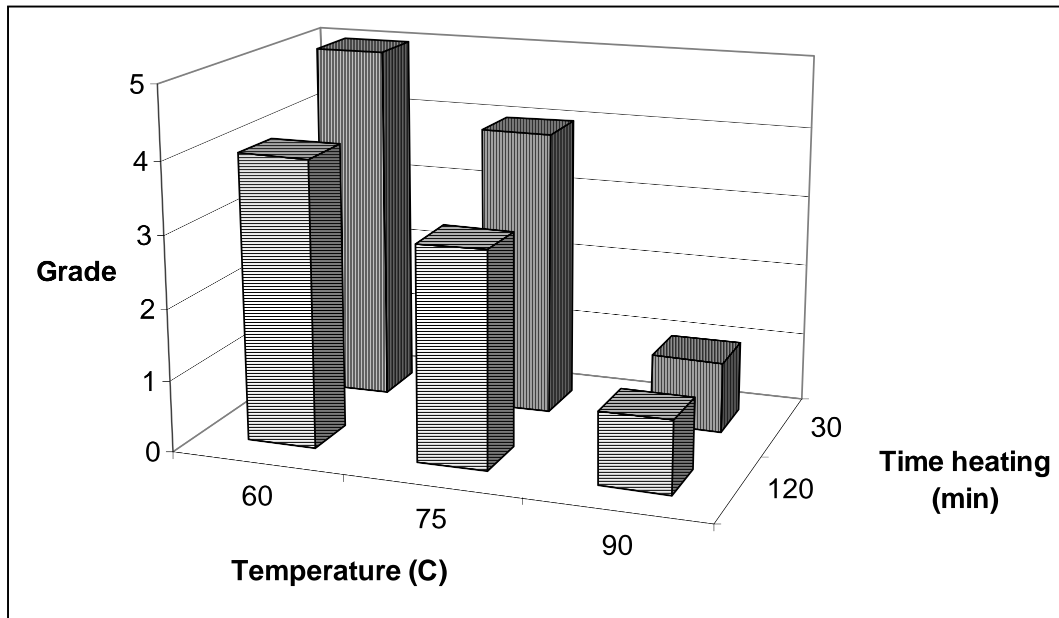


Figure 22. Wood samples ontaminated with 0,5 ml H.salinarum (10^8 CFU/ml) as a contaminant, incubated in 120 minutes at room temperature, and then heated at 60, 75 and 90°C in 30 (II) and 120 (=) minutes. The samples were heated with moist heat.

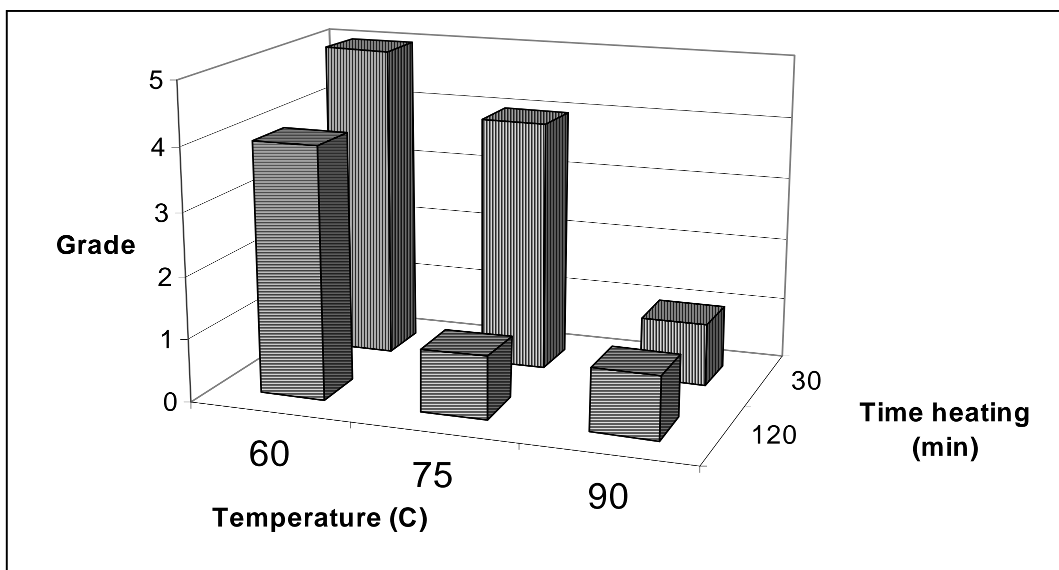


Figure 23. Wood samples with 0,5 ml H.salinarum (10^8 CFU/ml) added as a contaminant, incubated in 120 minutes at room temperature, and then heated at 60, 75 and 90°C in 30 (II) and 120 (=) minutes. The samples were heated in dry heat.

3.4.3 Incubation of samples in 7 days

Figure 24 shows results from experiment with 120 minutes incubation of wood samples, then heating at 60, 75 and 90°C in 30 and 120 minutes. The figure comprehends moist and dry heat, because there was no difference between the samples.

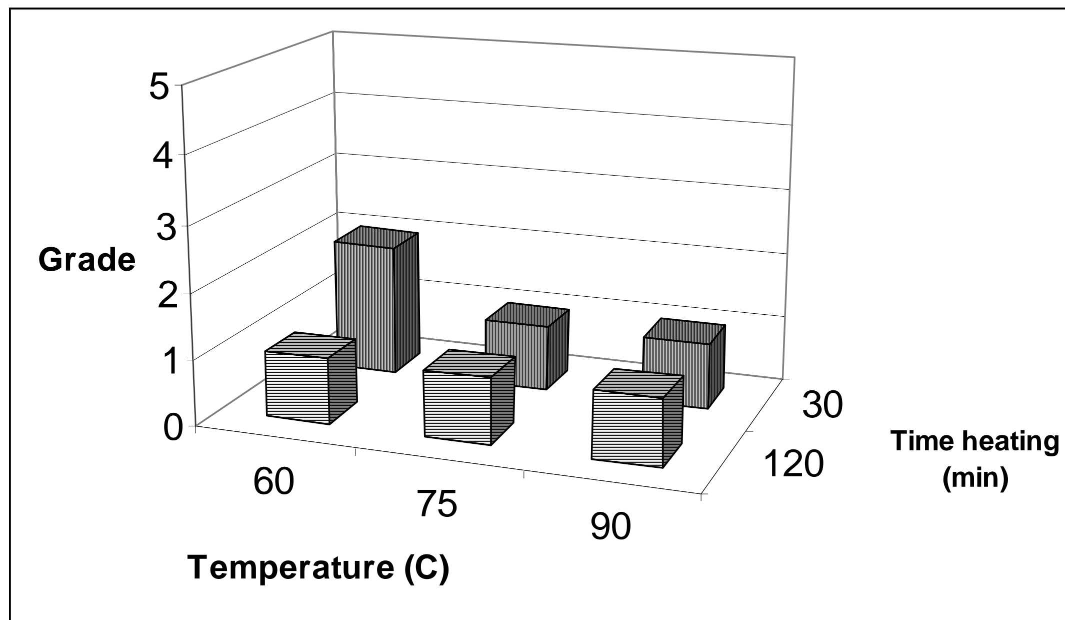


Figure 24. Wood samples with 0,5 ml H.salinarum (10^8 CFU/ml) as a contaminant, incubated in 120 minutes at room temperature, and then heated at 60, 75 and 90°C (dry and moist heat) in 30 (II) and 120 (=) minutes.

Figures 20 - 24 show that samples with *H.salinarum* as a contaminant, incubated in 30 and 120 minutes before heating, heating at 90°C in minimum 30 minutes, was sufficient to obtain "grade 1". When the time of incubation was prolonged to 7 days, 60°C in 120 minutes was sufficient to obtain "grade 1". The results from the contact method show a good coherence with the results from the swabbing method. *Results of the figure 20- 24 are presented in a table in appendix 6.*

3.5 Experiment with salted fish

In this experiment, we have studied the risk of crosscontamination from wood to salted fish by putting a sterile piece of salted fish on a wooden surface contaminated with *H.salinarum*. First, the wooden samples where contaminated with *H.salinarum* and incubated in 37°C in 30 minutes, 120 minutes and 7 days. Secondly, the wooden samples where heated (60, 75 and 90°C in 30 and 120 minutes). After the heating, a sterile piece of salted fish was put on each wooden sample. Then the samples (wood and salted fish) were put in a plastic box and incubated in 37°C for one month. The results are shown in table 3.

Table 3. Detection of H.salinarum (CFU/g) in salted fish after one month of incubation at 37°C in direct contact with a wooden sample. The wooden sample was contaminated with 0,5 ml H.salinarum (10^8 CFU/ml).

Heating temperature (C°)	**Time of heating (min)**	**Moist heat (+) / Dry heat (-)**	**Time of incubation in 37 C° before heating**		
			30 min	120 min	7 days
			H.salinarum (CFU/g)		
60	30	+	<1	> 300	<1
		-	<1 / 1 *)	> 300	<1
	120	+	<1	1	<1
		-	<1	> 300	<1
75	30	+	<1	<1	<1
		-	<1	<1	<1
	120	+	<1	<1	<1
		-	<1	<1	<1
90	30	+	<1	<1	<1
		-	<1	<1	<1
	120	+	<1	<1	<1
		-	<1	<1	<1

*) Results of two parallels

According to table 3, incubation time of 7 days for wooden samples containing *H.salinarum* (before putting the salted fish on), give no detection of *H.salinarum* when analysing the salted fish. When decreasing the time of incubation to 120 minutes, heating at 75°C in 30 minutes was required to obtain an acceptable result; no detection of *H.salinarum* in the salted fish (Anon, 1997). When the time of incubation was 30 minutes, heating of the wooden samples at 60°C in 120 minutes was required to obtain no detection of *H.salinarum*.

Some of the samples with salted fish became too dry during the incubation of 4 weeks. Due to this, the contact between the salted fish and the wooden surface became uneven. This has to be considered when comparing these results with the results from the swabbing and contact method.

4 CONCLUSION

4.1 Main conclusion

In general, these experiments show that heat treatment can be used as a method to reduce the number of microorganisms on a wooden surface to an acceptable level. The heating conditions (temperature, time) must be adjusted to the microorganisms that are expected to be present in the product and environment. Moist heat has more penetrating power than dry heat, and produces a faster reduction of the number of living organisms at a given temperature (Madigan *et al*, 2000).

The time of incubation of the contaminated sample before heating is of vital importance in order to detect the microorganisms. Increased incubation time involves less detection of microorganisms.

4.2 Methods for recovery

4.2.1 Swabbing method

<u>Microorganisms isolated from cod</u>
For samples with microorganisms isolated from cod added as a contaminant, incubation time up to 120 minutes, heat treatment using 100°C (moist heat) in minimum 150 minutes is required to obtain “grade 1”. When incubating the samples in 7 days, 120 minutes of heating at 100°C (moist heat) is required to obtain “grade 1”.

<u>H.salinarum</u>
For samples with *H.salinarum* added as a contaminant, incubation time in 30 minutes, heating treatment using 75°C in 60 minutes, moist heat is sufficient to obtain “grade 1”. When the samples were incubated in 120 minutes, heat treatment using 60°C, moist heat in 90 minutes was required to obtain “grade 1“. When incubating the samples in 7 days, “grade 1” was obtained after 60°C in 60 minutes, moist heat.

4.2.2 Contact method

<u>Microorganisms isolated from cod</u>
Samples incubated in 30 minutes with microorganisms isolated from cod, did not obtain “grade 1” even after heating up to 90°C. When prolonging the time of incubation to 120 minutes and 7 days, 90°C in 120 minutes was required to obtain “grade 1”. At this time and temperature condition, no difference between dry and moist heat was observed.

By using the contact method, less *H.salinarum* was obtained on the petri dish compared to the swabbing method.

<u>*H.salinarum*</u>
When the incubation time is 30 or 120 minutes, heating at 90°C in 30 minutes is sufficient to obtain “grade 1”. When the incubation time was increased to 7 days, 60°C in 120 minutes was required to obtain “grade 1”.

By using the contact method, more microorganisms was obtained on the petri dish compared to the swabbing method.

4.3 Experiments with salted fish

After incubation of the samples (wood and salted fish together) in one month at 37°C, analysis of *H.salinarum* in the salted fish was performed. *H.salinarum* was not detected in salted fish that had been in contact with wooden samples, which had been incubated in 7 days with *H.salinarum* and heated (60, 75 or 90°C). In fact, *H.salinarum* was detected only in the salted fish that had been in contact with contaminated wooden samples incubated in 30 or 120 minutes, heated at 60°C in 120 minutes.

When comparing the results obtained from the swabbing method, we are able to detect the *H.salinarum* on the samples incubated in 30 minutes and heated at 60°C in 120 minutes. When analysing the corresponding sample with salted fish, *H.salinarum* was not detected. This indicates that *H.salinarum* detected on the wooden surface will not necessarily contaminate the salted fish.

When temperature is kept low in the salt fish industry, the probability of having problems with *H.salinarum* is quite low. If there are problems with the cooling system, or in hot summers, there may be a problem with growth of *H.salinarum*. It is expected that low numbers of *H.salinarum* is present in the salt that is used to produce salted fish. Therefore, the challenge is to minimise the risk for temperature rise. In our experiments the "worst case" is simulated; the high numbers of microorganisms and optimum growth conditions for *H.salinarum*. Since *H.salinarum* was not detected after heating, it is realistic that the microorganism would not be detected on the wood in the industry either.

5 ACKNOWLEDGEMENTS

We would like to thank Guro Pedersen, Reidun Dahl, Olaug Taran Skjerdal and Margrethe Esaiassen at the Norwegian Institute of Fisheries and Aquaculture Ltd. for performing the experiments in the laboratory and useful help. Aven Treindustrier A/S for providing the samples and Birna Guðbjörnsdóttir at Icelandic Fisheries Laboratories for input during our work.

6 REFERENCES

Anon, 1999. Treteknisk Håndbok. Norsk Treteknisk Institutt. Teknisk småskrift nr 33. 2. Utgave. Oslo.

Anon, 1997. Kvalitetsforskrift for fisk og fiskevarer. Fiskeridirektoratet. Avdeling for kvalitetskontroll. Bergen. Norge.

Anon, 1992. Mikrobiologiske retningsliner for næringsmiddel. Statens Næringsmiddeltilsyn. 1. utgave. Oslo.

Hobbs G., 1991, Fish: Microbiological spoilage and safety. Food Science and Technology Today, 5, 166-173.

Larsen, H. 1984. Family V. Halobacteriaceae. In Bergeys manual of systematic bacteriology. 261 – 267, Vol 1. Williams & Wilkins.

Lorentzen, G. *et al*. 2000. Wood in Food. Measuring methods. Partial report 1. Fiskeriforskning. Tromsø.

Madigan, M.,Martinko, J,M. And J. Parker. Brock Biology of Microorganisms. Prentice Hall International Inc. 9th edition.

van Klavern, FW. and Legendre, R. 1965. Salted cod. Borgstrom G (ed) Fish as food, Vol III Processing: Part 1, Akademic Press, London

7 APPENDIX

App 1: Halofile og osmofile mikrober (rød og brunmidd). Bestemmelse i fullsaltede fiskeprodukter (in Norwegian). 5 pages.

App 2: A modified grading system for interpreting results of swabbing and contact method.

App 3: Detection (grade) of microorganisms isolated from cod on a wooden surface after heating at 60, 75 and 90°C by using the swabbing method.

App 4: Detection (grade) of *H.salinarum* on a wooden surface after heating at 60, 75 and 90°C by using the swabbing method.

App 5: Wood samples contaminated with microorganisms isolated from cod, incubated in room temperature in 30 minutes, 120 minutes and 7 days, heated at different conditions (time, temperature and moist heat) and then analysed with the contact method.

App 6: Wood samples contaminated with *H.salinarum*, incubated in room temperature in 30 minutes, 120 minutes and 7 days, heated at different conditions (time, temperature and moist heat) and then analysed with the contact method.

Appendix 1

Halofile og osmofile mikrober (rødmidd og brunmidd). Bestemmelse i fullsaltede fiskeprodukter.

1. Formål og anvendelsesområde

Metoden kan anvendes for å påvise rødmidd og brunmidd i saltfisk.

Nedre grense for deteksjon er 100 kim av aktuell mikrobe per gram prøve. Bemerk at det kan være store variasjoner i mikrobeinnhold på ulike deler av fisken.

2. Definisjoner og epidemiologiske aspekter

2.1 Rødmidd

Rødmidd er en tradisjonell bransjebetegnelse på synlig vekst av ekstremt halofile bakterier på fullsaltet fisk. Bakteriene tilhører familien Halobacteriaceae som omfatter kokker (Halococcus) og staver eller skiveformede (engelsk: disc-shaped) bakterier (Halobacterium). Halobacteriaceae tilhører klassen Archaeobacteria, og er strikt halofile. De har annerledes celleveggoppbygning enn de fleste andre bakterier, men gir Gram negativ reaksjon ved modifisert Gram test med KOH. De fleste arter av rødmidd er ubevegelige og strikt aerobe. Noen krever bare 8 % NaCl for å vokse, men i de fleste tilfellene behøves 17-23 % NaCl for god vekst. Koloniene har ulike fargenyanser av rødt; rosa, orange/rød, skarlagensrød eller rødfiolett mens andre kan være fargeløse. Halococcus og Halobacterium påvises ofte i samme materiale.

2.1.1 Halobacterium

Halobacterium er stav- eller skiveformede bakterier. De fleste isolerte stammene behøver minst 15 % NaCl for å vokse, og de vokser best ved 20 - 26 % NaCl. De kan også vokse i mettede saltløsninger; 29 % NaCl. Optimumstemperaturen for vekst er 40 - 50 °C. Disse organismene forårsaker blant annet rød misfarging på saltfisk (norsk bransjeuttrykk ”rødmidd” eller engelsk ”pink”).

Organismene har et komplekst næringskrav; de trenger flere aminosyrer for å vokse. De kan også utnytte aminosyrer som energikilde. Halobacterium vokser sakte; en generasjonstid på 3-6 timer er det beste som er oppnådd i laboratorieforsøk.

Slekten Halobacterium omfatter artene:
Halobacterium salinarium, Halobacterium volcanii, Halobacterium saccharovorum, Halobacterium vallismortis, Halobacterium pharaonis

2.1.2 Halococcus

er kokker som opptrer enten parvis, i tetraeder, sarcinapakker eller i irregulære klaser. Halococcus krever minst 15 % NaCl for å vokse, og den vokser optimalt ved 20 - 26 % NaCl. Den kan vokse fra 8 til 55 °C, temperaturoptimum er 30- 37 °C. Bakterien vokser sakte (generasjonstid på 14 timer), selv under optimale vekstvilkår. Vekst av Halococcus indikeres ved synlige røde kolonier.

Slekten Halococcus består av en aktuell art: Halococcus morrhuae

2.2 Brunmidd

Brunmidd (engelsk ”dun”) er en tradisjonell betegnelse på forekomst av brune kolonier (1-2 mm i diameter) på saltfisk. Brunmidd er en encellet sopp (*Wallemia sebi*, tidligere *Sporendonema epizoum*). Brunmidden er strikt aerob. Den er osmofil/xerofil, og vokser på substrat som inneholder 5 - 26 % NaCl, 20 % sukrose, og 20 % glyserol. Den vokser ved 5 - 37 °C, og ved pH 4–8. De optimale vekstvilkår er ved vannaktivitet tilsvarende 10 - 15 % NaCl, 75 % relativ fuktighet og 25 °C. Veksten stimuleres av lys. Soppcellene er klubbeformet. Fargen på pigmentet endres med saltinnholdet; lavt saltinnhold gir sjokolade brune kolonier, medium saltinnhold gir lysebrune kolonier og et høyt saltinnhold gir grønn/brune kolonier.

3. Referanser

3.1 NMKN 5, 1994, Handbok för mikrobiologiska laboratorier. Handledning för intern kvalitetskontroll av analysarbetet. 2. utgave

3.2 NMKN 91, 1988: Forbehandling av levnedsmidler til mikrobiologisk undersøgelse.

4. Prinsipp

Rød- og brunmidd kan påvises ved utsæd på egnede agarmedier. Prøver for rødmidd inkuberes lyst ved 37 °C i 2 uker, mens prøver for brunmidd inkuberes lyst ved 20 – 24 °C (romtemperatur) i 2 uker eller mer. I de fleste arter danner synlige kolonier innen 4 dager. Både rød- og brunmidd kjennetegnes ved at de gir pigmenterte kolonier.

5. Fortynningsvæske, substrater

5.1 Fortynningsmedium for rødmidd

Salt (NaCl)	250 g
Pepton	1.0 g
Destillert vann	1000 ml

Fortynningsmediet autoklaveres ved 121 °C i 20 minutter. Etter sterilisering skal pH i bruksferdig løsning være 7,4 +/- 0,2 målt ved 20-25°C.

5.2 Fortynningsmedium for brunmidd

Salt (NaCl)	75 g
Pepton	1,0 g
Destillert vann	1000 ml

Autoklaver fortynningsmediet ved 121°C i 20 minutter etter at ingrediensene er løst. Etter sterilisering skal den bruksferdige løsningen ha pH 5.6 ± 0.2 målt ved 20 - 25°C.

5.3 Rødmidd – medium

Kasaminosyrer	7,5 g
Gjærekstrakt	10,0 g
Natriumklorid (NaCl)	250,0 g
Magnesiumsulfat ($MgSO_4$ x $7H_2O$)	20,0 g
Natriumcitrat	3,0 g
Kaliumklorid	2,0 g
Jernsulfat ($FeSO_4$ x $7H_2O$)	0,05 g
Mangansulfat ($MnSO_4$ x H_2O)	0,20 mg
Agar	20,0 g
Destillert vann	1000 ml

Løs opp stoffene under omrøring og juster pH. Tilsett agar, og autoklaver ved 121°C i 20 minutter. Etter sterilisering skal pH i bruksferdige substrat være 7,4 ± 0,2 målt ved 20 - 25°C.

5.4 Brunmidd - medium

5.4.1 Alternativ I (Modifisert Vaisey medium)

Kasiton	2,5 g
Magnesiumsulfat ($MgSO_4$)	0,2 g
Jern (II) sulfat	0,02 g
Dikaliumhydrogenfosfat	1,0 g
Natriumklorid	75,0 g
Glucose	9,0 g
Agar	25,0 g
Destillert vann	1000 ml

Løs opp alle stoffer unntatt magnesiumsulfat og jernsulfat. Løs opp 2 g magnesiumsulfat og 0,2 g jernsulfat i 10 ml destillert vann (stamløsning). Pipetter ut 1 ml av stamløsningen og tilsett mediet. Juster pH og autoklaver ved 121 °C i 20 minutter. Etter sterilisering skal pH i bruksferdig medium være 7,2 +/- 0,2 målt ved 20-25°C.

5.4.2 Alternativ II (Modifisert dichloran – glyserol (DG 18) Agar Base)

Bemerk at mediet er noe forskjellig fra DG 18 – mediet som er beskrevet i NMKLs metodeforslag nr. 98, 3. opplag 1995.

Pepton	5,0 g
Glukose	10,0 g
Dikaliumhydrogensulfat	1,0 g
Magnesiumsulfat ($MgSO4x7H_2O$)	0,5 g
2,6-Diklor-4-nitroanilin (Dichloran)	0,002 g
Agar	15,0 g
Glyserol (p.a.)	220,0 g
Kloramfenikol	0,10 g
Destillert vann	1000 ml

Løs opp alle ingrediensene, glyserol og kloramfenikol til de løses. Bruk maksimalt 500 ml medium (målt før tilsats av glycerol) i hver kolbe. Juster pH og tilsett glyserol. Mediet steriliseres ved autoklavering ved 121°C i 20 minutter. Kjøl ned til ca 50°C og tilsett steril kloramfenikolløsning. Hell mediet over i sterile pestriskåler. Etter sterilisering skal pH i det bruksferdige substrat være 5.6 ± 0.2 målt ved 20 - 25°C. Dehydrert basismedium og steril kloramfenikol supplement finnes kommersielt tilgjengelig.

6. Apparatur og glassutstyr

Normal utrustning for et mikrobiologisk laboratorium.

Termostatskap med lys, 37,0 ± 1,0 °C.

7. Prøveuttak

Foreta prøveuttak med steril skalpell på overflaten av saltfisken etter vanlige bakteriologiske prinsipper, slik at prøven blir mest mulig representativ. Prøven kan transporteres før analyse. Påse at prøven er godt innpakket.

8. Fremgangsmåte

8.1 Forbehandling

Utfør forbehandling og fortynning av prøvene i samsvar med NMKL-metode nr. 91. Bemerk at fortynningsvæsken i denne metoden er forskjellig fra den som er beskrevet i nr 91.

8.2 Utsæd, inkubasjon og avlesing

8.2.1 Rødmidd

Overfør 0,1 ml av homogenisatet til en petriskål med ferdigstøpt rødmidd medium. Dersom det ikke er synlig rødskjær eller kolonier av rødmidd på fisken er det ikke nødvendig å lage fortynningsrekke. Stryk prøvematerialet inn i mediet med en steril og avkjølt glasstav. Petriskålene pakkes inn i plastikkposer og inkuberes lyst ved 37 °C. Vekst indikeres ved utvikling av røde kolonier. Rødmiddkolonier kan være synlige allerede etter 4-7 døgn. Dersom det ikke er synlige kolonier etter 2-3 uker regnes prøven som negativ.

8.2.2 Brunmidd

Overfør 0,1 ml av homogenisatet til en petriskål med ferdigstøpt brunmidd medium. Dersom det ikke er brune prikker på fisken er det ikke nødvendig å lage fortynningsrekke. Stryk prøvematerialet inn i mediet med en steril og avkjølt glasstav. Petriskålene pakkes inn i plastikkposer og inkuberes lyst ved ca 20 °C / romtemperatur. Vekst indikeres ved utvikling av lysbrune/beige kolonier. Brunmiddkolonier kan være synlige allerede etter 3-4 døgn. Dersom det ikke er synlige kolonier etter 1-2 uker regnes prøven som negativ.

9. Konfirmering

9.1 Rødmidd

Vekst på rødmiddmediet men ikke på brunmiddmediet.

9.2 Brunmidd

Vekst på brunmiddmediet men ikke på rødmiddmediet.

10. Resultat

Tell antall pigmenterte kolonier, hhv røde og brune, og regn ut antall kolonier og angi resultatets kolonitall per gram prøve eller per cm^2 overflate. Upigmenterte kolonier regnes ikke med.

11. Litteratur

Referanser for mediene i denne oppskriften har vært følgende:

Rødmiddmedium

DSMZ nr 97 (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH)

Brunmidd medium med 7,5 % NaCl

Burgos J, Sala FJ, Lopéz A, 1973, Quinones and respiratory activity in

Spoendonema epizoum. Phytochemistry 12:1201-1206.

Brunmiddmedium med Dichloran-glycerol (DG18) agar base

1. Hocking A. D. And Pitt J. I. 1980. J. Appl. & Env. Microbiol. 39. 488 – 492.

2. Beckers, H. J., Boer, E., van Eikelenboom, C., Hartog, B. J., Kuik, D., Mol, N., Nooitgedagt, A. J., Northolt, M., O. & Samson, R. A. 1982. Intern. Stand. Org. Document ISO/TC34/SC9/N151

12. Referenter for metoden

Denne NMKL-metoden er utarbeidet av Grete Lorentzen, Guro Pedersen og Olaug Taran Skjerdal, Fiskeriforskning, Tromsø, Norge.

Appendix 2

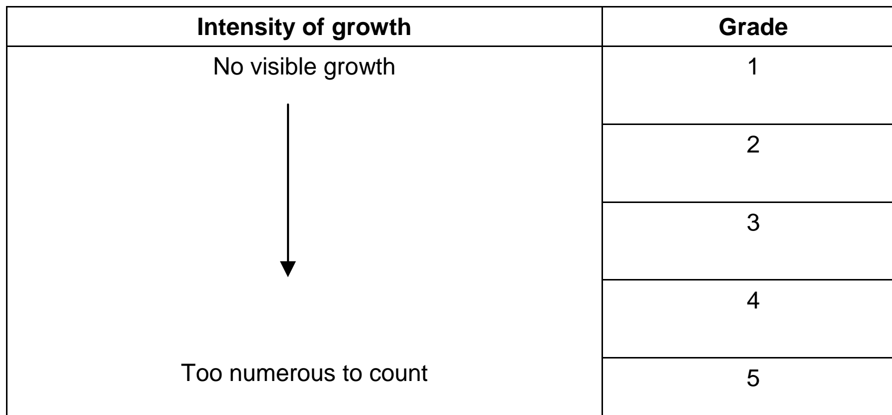
A modified grading system for interpreting results of swabbing and contact method

Swabbing method

CFU[1] on a petridish	Grade		
	MICROORGANISMS ISOLATED FROM COD		*Halobacterium salinarum*
> 1			1
1 – 25	1	*	2
25 – 50	2	**	3
50 – 100	3	***	4
100 – 200	4	****	5
200 – 300	5	*****	6
> 300	6	******	7

1) Colony Forming Units
We used both a scale from 1 to 6 or asterisks (*) to indicate the number of colony forming units.

Contact method



Intensity of growth	Grade
No visible growth	1
↓	2
	3
	4
Too numerous to count	5

Appendix 3

Detection (grade) of microorganisms isolated from cod on a wooden surface after heating at 60, 75 and 90°C by using the swabbing method.

Heating temperature (⁰C)	Time heating (min)	Moist heat (+) / dry heat (-)	Time of incubation in 37⁰C before heating		
			30 min	120 min	7 days
			Grade		
60	30	+	6	6	6
		-	6	6	6
	60	+	6	6	6
		-	6	6	6
	90	+	6	6	6
		-	6	6	6
	120	+	6	6	6
		-	6	6	6
75	30	+	6	6	6
		-	6	6	5
	60	+	6	6	5
		-	6	6	4
	90	+	6	6	5
		-	6	6	5
	120	+	4	6	5
		-	6	6	4
90	30	+	5	6	6
		-	6	6	6
	60	+	4	5	6
		-	6	6	4
	90	+	2	3	4
		-	5	6	2
	120	+	2	3	4
		-	4	4	4

Detection (grade) of *h.salinarum* on a wooden surface after heating at 60, 75 and 90°C by using the swabbing method.

Heating temperature (ºC)	Time heating (min)	Moist heat (+) / dry heat (-)	Time of incubation in 37ºC before heating		
			30 min	120 min	7 days
			Grade		
60	30	+	4	4	2
		-	6	5	2
	60	+	4	3	1
		-	5	5	1
	90	+	5	1	1
		-	3	1	1
	120	+	4	1	1
		-	4	1	1
75	30	+	4	1	1
		-	1	1	1
	60	+	1	1	1
		-	1	1	1
	90	+	1	1	1
		-	1	1	1
	120	+	1	1	1
		-	1	1	1
90	30	+	1	1	1
		-	1	1	1
	60	+	1	1	1
		-	1	1	1
	90	+	1	1	1
		-	1	1	1
	120	+	1	1	1
		-	1	1	1

Appendix 5

Wood samples contaminated with microorganisms isolated from cod, incubated in room temperature in 30 minutes, 120 minutes and 7 days, heated at different conditions (time, temperature and moist heat) and then analysed with the contact method. Grade “1”is no visible growth and grade “5” is to numerous to count.

Heating temperature (ºC)	Time of heating (min)	Moist heat (+) / Dry heat (-)	Time of incubation in room temperature before heating		
			30 min	120 min	7 days
			Grade		
60	30	+	5	5	5
		-	5	5	5
	120	+	4	4	4
		-	4	4	3
75	30	+	5	5	5
		-	4	5	4
	120	+	2	3	3
		-	2	3	1
90	30	+	2	3	1
		-	4	3	3
	120	+	2	1	1
		-	2	1	1

Appendix 6

Wood samples contaminated with H.salinarum, incubated in room temperature in 30 minutes, 120 minutes and 7 days, heated at different conditions (time, temperature and moist heat) and then analysed with the contact method. Grade “1”is no visible growth and grade “5” is to numerous to count.

Heating temperature (ºC)	Time of heating (min)	Moist heat (+) / Dry heat (-)	Time of incubation in 37ºC before heating		
			30 min	120 min	7 days
			Grade		
60	30	+	3	5	2
		-	5	5	2
	120	+	3	4	1
		-	5	4	1
75	30	+	3	4	1
		-	2	4	1
	120	+	2	3	1
		-	1	1	1
90	30	+	1	1	1
		-	1	1	1
	120	+	1	1	1
		-	1	1	1

Fiskeriforskning

Tromsø (head office)
Muninbakken 9-13, Breivika
P.O.B 6122
NO-9291 Tromsø
Norway
Tel.: +47 77 62 90 00
Fax: +47 77 62 91 00
E-mail:post@fiskeriforskning.no

Bergen
Kjerreidviken 16
NO-5141 Fyllingsdalen
Norway
Tel.: +47 55 50 12 00
Fax: +47 55 50 12 99
E-mail: office@fiskeriforskning.no

Internet: www.fiskeriforskning.no

ISBN 82-7251-454-0
ISSN 0806-6221