

SLAGTERIERNES FORSKNINGSinSTITUT  
4. august 1964

KONSERVES-MIKROBIOLOGI  
Manuskript nr. 284E

Simplified Bacteriological Control Methods in the Canning Industry  
(preliminary information)  
by

Jørgen Baltzer, Veterinarian

The Danish Meat Research Institute, Roskilde, Denmark

Summary

The paper deals with the bacteriological problems in connection with manufacturing of heat-processed canned foods with a satisfactory keeping quality and organoleptic quality. It describes a simple method for routine control with the content of heat-resistant clostridium spores. Furthermore, two simple methods are mentioned for the control of total bacterial numbers in raw materials and in raw-mix before heat-processing. These methods are worked out in such a way that the control can be performed in the factory by persons with no bacteriological education and without laboratory facilities. Through these methods it is possible for the factories to carry out a daily bacteriological control of the proces in a practical and economical way.

SLAGTERIERNES FORSKNING SINSTITUT  
4. august 1964

KONSERVES - MIKROBIOLOGY  
Manuskript nr. 284 E

### Introduction

The main purpose in heat processing of food in cans is to secure killing of all living microorganisms being able to spoil the product or being a danger to public health.

The demand to the manufacturer in connection with the keeping quality of canned products is: Swelling must not take place in more than 1 tin per 10.000 tins. Furthermore, the demands regarding keeping quality seem to increase.

With regard to security of public health the demands are even more strict, the demand being that not more than 1 tin out of  $10^{12}$  tins will give risk for botulism. Precautions to meet this demand have been of especial interest after the many cases of botulism in recent years.

The heat treatment normally given products like luncheon meat will kill all vegetative cells, while the amount of heat resistant spores of putrefactive (anaerobe) bacteria only will be reduced by a factor of 100. This means that the heat-treatment of a minced meat product, containing 1000 heat resistant spores per can, results in a finished product containing about 10 spores per can. In this lot 100 % swelling will be expected. If there is only 10 spores per can before the processing, the finished product will consequently contain 0.1 spore per can. So 90 % of the cans will in any circumstances be safe. It is therefore evident that the heat-processing alone cannot guarantee a satisfying keeping quality, as mentioned above. Safety must mainly be obtained by means ensuring a small amount of heat-resistant spores in the product before heat-processing.

This can only be obtained by checking all the ingredients, which are used in the product and exclude those which induce a significant amount of spoilage organisms.

Presumably such a controlsystem will also give the best security against occurrence of *Clostridium botulinum* in canned products as the chance of occurrence of a can containing *Clostridium botulinum* will be reduced when the amount of putrefactive anaerobes as a whole is reduced.

SLAGTERIERNES FORSKNINGSinSTITUT  
4. august 1964

KONSERVES - MIKROBIOLOGI  
Manuskript nr. 284 E

A very important fact in connection with the organoleptic quality of processed meat is this: Raw-mix with a content of living and dead bacteria (cells) more than 10-20 mill per gram gives with great probability a finished product with unpleasant taste or off-flavour.

Furthermore, abnormal texture is often seen in products with so high an amount of bacteria, i.e. soft spots are found, or the whole meat block has a soft consistency.

The heat processing involves that this quality deterioration does not continue, but it is unable to influence the mentioned organoleptic quality faults caused by microorganisms, which have taken place already.

A routine bacteriological control covering 1) the quality of the raw material and 2) the quality of the product just before the heat-processing, 3) the hygiene in the factory etc., may disclose the possibility of a faulty production at a very early stage. The cause of the faults can be investigated and controlled with considerable greater success than now where control in most of the factories only include testing of the finished product by incubation at elevated storage temperatures. The chances for fault-finding by this last mentioned procedure has often been mentioned.

The work in connection with a routine bacteriological control of all the ingredients in the products will be very great. The factories' laboratories are very often overloaded and it will hardly be possible in any factory to carry out such a control if classical bacteriological methods must be used.

In the following are described in principle some control methods, which are developed by the Danish Meat Research Institute. These methods are made very simple and do not demand laboratory facilities, but can be performed directly in the factory. These methods are accurate enough for the purpose, and can be performed by persons without any bacteriological education.



SLAGTERIERNES FORSKNING SINSTITUT  
4. august 1964

KONSERVES - MIKROBIOLOGI  
Manuskript nr. 284 E

I Methods for control with anaerobic heat-resistant spores (Clostridium spores)

Background: Swelling in heat-processed canned food appears, when spores of anaerobic putrefactive organisms have survived the treatment, germinate and begin to grow in the product. During the growth the contents of the can is spoiled and the cells produce gas. The gas produced makes the can swell and the pressure in the can may be so great that the can bursts and the contents spill out. However, spores have a typical inclination for delayed germination even in food, which are suitable for growth. In canned products, this delayed germination (dormancy) is very distinct because the product contains salt and nitrite. For this reason a long incubation period is necessary when testing the finished product.

Principle: a) By a certain heat treatment (temperature/time) a certain percentage of existing spores in the examined material are killed. The spores therefore can be counted by heat treatment of minced meat samples at lengths are on different lengths of time, at ex. 100°C in cans. The cans will swell after an incubation at elevated temperature, if there are surviving spores present.

b) The inhibitory effect of salt and nitrite on germination can be reduced by dilution with water.

Procedure:

Examination of uncooked minced meat (raw mix): portions of 25 grams each of minced meat is weighed out into 10 empty 1/8 kg cans. Exact weighing is necessary. The cans are filled up with lukewarm water, are closed and shaken carefully. All the cans are then placed in boiling water in a waterbath or open autoclave. The water must boil again as quickly as possible and be kept boiling (100°C-control with thermometer). After precisely 15 minutes, 3 cans are removed and cooled in running cold water. 30 minutes after the boiling began another 3 cans are removed, and after 45 minutes 3 cans again. The last can is taken out after 60 minutes. The cans are cooled in running cold water for 15-30 minutes, then carefully marked with the date, contents, and minutes in boiling water. Afterwards they are placed in thermostat at 37°C. The cans are inspected once a day and carefully shaken. After a incubation period of 5 days, the number of swelled cans are counted. As guiding standard it can be stated that more than 6 cans must swell at 37°C. This will correspond to about 0.5-1 spore per gram in the material examined.

SLAGTERIERNES FORSKNINGSINSTITUT  
4. august 1964

KONSERVES - MIKROBIOLOGI  
Manuskript nr. 284 E

### Examination of the raw materials

a) Raw material: 25 grams of the fresh raw material, beef-trimmings, rind, fat, pork meat etc. are weighed into of 10 1/8 kg cans. Before the weighing the meat is cut in small pieces with scalded utensils. The cans are filled up with water and treated as described.

Example of a result:

	15'	30'	45'	60'
Beef	+++	+++	+++	+
Pork	++	++	++	-
Rind (boiled)	+++	++	++	-
Fat	++	++	++	-

In the example the beef contains many heat resistant spores and should be omitted in the production.

b) Additives: An extended examination must also include spices, flour, etc. 1 gram of additive is weighed into a can and 25 grams of diced meat are added. Before cutting, the meat must be cut clean and scalded for 10 seconds in boiling water. The cutting must be performed with scalded utensils. All this to secure that the spores in the cans originate from the additives. The procedure and the readings are as mentioned above.

Remarks. The method is developed by making many systematical examinations of spore-free raw materials and minced meat to which a known amount of clostridium-spores have been added before the boiling. The spore suspension (No. 93) which is produced using current methods with a (*Clostridium sporogenes*) isolated from swelled luncheon meat, is stored in M/15 (phosphat-buffer). The heat resistance in this strain was normal. (comp. to PA 3679)

Then different combinations of spore numbers per gram/meat-water ratio / time at 100°C / time of incubation at 37°C, were investigated until the right combination of these factors was found, i.e. the combination which in a reasonable time (120 hours) will give swelling in some cans but not all of 10 cans, when the spore number is about 0.5 - 1.0 per gram in the investigated material. A spore number of this order of magnitude is rather high, but with normal treatment there will be a low swelling percent. It is intended, when there is a need for it, to develop a more sensitive method according to the same principles.

...



SLAGTERIERNES FORSKNING SINSTITUT  
4. august 1964

KONSERVES - MIKROBIOLOGI  
Manuskript nr. 284 E

Using the above mentioned method for control of spores and with presence of about 0.5 - 1.0 spore per gram we following results were obtained (10 cans - 120 hours/37°C):

(number of swelled cans)

	<u>Minced meat</u>	<u>Fresh trimmings</u>
	3/10	1/10
	6/10	2/10
	6/10	3/10
	6/10	5/10
	7/10	6/10
	8/10	6/10
average	6/10	4/10

Minced meat with 10-20 spores per gram gives 100 % swelling in 96 hours.

White pepper with 1000 spores per gram gives 100 % swelling in 48 hours.

White pepper with 1 - 10 spores per gram gives 20 - 30 % swelling in 120 hours, etc.

This method using cans is probably the only possible technique when working with investigation of heat resistant spores in natural environment and at a natural level.

As the spores in e.g. luncheon meat mix very often are irregular dispersed the possibility of finding the spores in the sample is much better with this method than the standard methods. Furthermore this method uses the properties in the spores, which cause the fault in the product.

II

#### Methods of controlling the quality of trimmings and raw mix (minced meat)

As mentioned: a raw mix which contains more than 10-30 million living bacteria per gram corresponding to 60-100 million living and dead bacteria per gram is likely to give finished products with a poor taste. Due to the many bacteria the raw mix contains a lot of off-flavour products from the bacterial decomposition which dominate the taste of the meat. Often the finished product will thus taste like raw potatoes, like burned rind, etc. Experiments made this spring at the Danish Meat Research Institute have shown a direct relation between the contents of microorganisms and the poor taste. The judgement: "unacceptable" is given by the tastepanels, when the numbers are of the size mentioned above. Therefore it would be reasonable if the factories aim at making a raw mix of a bacterial count of less than 10 million per gram. In order to do this, the trimmings used in the production must necessarily have a sufficiently low bacterial count both when arriving at the factory and when used.

SLAGTERIERNES FORSKNINGSinSTITUT  
4. august 1964

KONSERVES - MIKROBIOLOGI  
Manuskript nr. 284 E

In the following two methods with relation to these requirements will be described, i.e. a method to judge the quality of trimmings and a method to judge the raw mix.

Examination of trimmings:

Principle: Both methods are based on reduction of the blue dye resazurin.

To examine the quality of trimmings you use filter-paper impregnated by resazurin-solution. At a rate dependent upon the bacteriological quality of the meat the blue colour of the paper will gradually turn violet, red-violet and red, perhaps even a white-gray colour. The poorer the meat the quicker the colour change. (It has been observed that the colour may turn white-gray in less than two minutes).

#### Materials

Each examination requires 3 pieces of resazurinpaper (this is made by saturating a piece of circular Watman-filter-paper with a resazurin-solution, drying and cutting it into strips).

Method: From the portion of meat to be examined pieces of meat weighing about 1 kg in all are taken out. Only thawed pieces can be tested.

These pieces are put into a polyten-bag (for instance 28x44 cm). The bag is closed and from the outside the pieces are mixed well together so that that their surfaces rub one another. Thus a better distribution of the bacterial colonies is achieved.

With clean fingers the resazurin-papers are removed from the small bag in which they are stored, and wetted slightly in slowly running tap water. Superfluous water is thrown off and the paper is placed on the meat in the big plastic-bag; there must be a good contact between paper and meat surface. Then the bag is turned over so the meat rests on the meat rests on the resazurin-paper.

Having been on the meat for 1 minute, the paper is returned to the small plastic bag (avoid overlapping). The air in the bag is carefully squeezed out. The meat has not suffered and can be used normally.

...



SLAGTERIERNES FORSKNING SINSTITUT  
4. august 1964

KONSERVES - MIKROBIOLOGI  
Manuskript nr. 284 E

It is important that the paper contacted the meat well. This can easily be controlled by looking at the paper as soon as it is put in the small bag; where it has not touched the meat it will have a clearer blue colour than elsewhere.

During the examination, the bag with the paper strips must be kept in darkness and at 22-23°C.

The colour of the strips is controlled after 10, 30 and 60 minutes.

If the pieces of meat have been efficiently mixed in the bag the colour on the paper-strips will turn gradually and be well spread on the whole paper blue - violet - redviolet - clear rose-red - discolouration.

Results: Change to red colour or colourless in less than 10 minutes

10 minutes:	not acceptable
between 10-30 "	just acceptable
30-60 "	good quality
more than 60 "	good quality

#### Examination of raw mix

The natural colour of raw mix makes it difficult to fix the time for a change of colour of resazurin from blue to red. However, it is not difficult to see that the blue colour has vanished if after some time a new mix/resazurin mixture is made and compared with the first one.

Method: One part of raw mix is mixed with one part of lukewarm water in a plastic-bag or in a glass and is kneaded or shaken till you get a good mixture without lumps.

To one tube you add at once 20 drops of resazurin-solution (1 tablet BDH in 50 ml water) and you shake the tube. All tubes are placed in dark at 22-23°C.



SLAGTERIERNES FORSKNING SINSTITUT  
4. august 1964

KONSERVES - MIKROBIOLOGI  
Manuskript nr. 284 E

It must be considered whether there is more or less than 10 mg ascorbic acid per kg raw mix. With luncheon meat the adding of resazurin to tube 2 and 3 is done according to the following scheme:

Contents of ascorbic acid	1. tube	2. tube	3. tube
more than 10 mg/kg	0 min.	15 min.	45 min.
less than 10 mg/kg	0 min.	15 min.	55 min.

When tube no. 3 has received the solution and been shaken it's colour is immediately compared to that of tube no. 1 and 2.

Results: Tube 2 has the same shade of blue colour as tube 3 and tube 1 is still somewhat blue: Satisfying quality.  
Tube 2 is partly or completely discoloured and tube 1 is quite without shade of blue: unacceptable.

#### Final remarks

The methods thus described has been introduced to the Danish manufacturers of heat-preserved canned meats, and many of them have started a routine control with the processing.

The work with these methods is easily overseen, and the results are, even if not very accurate, very useful for a judgement of the bacteriological standard of the products involved, and it helps to secure that the products will be stable and acceptable.