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364

The Effect of Chlortetracycline in Canned Meat Products

by

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The present paper deals mainly with an experiment carried out at the Danish Meat Research Institute on the effect of chlortetracycline (CTC) on the keeping quality of luncheon meat.

The experiment was made to determine the effect of different treatments on the organoleptic quality of luncheon meat and can corrosion problems as well as the effect on bacterial counts and types in luncheon meat. The treatments included addition of CTC to the ham trimmings (3 levels), storage of the ham trimmings (3 levels), holding time before retorting (2 levels), and sterilizing values (2 levels). The experiment was carried out as a full replicate factorial experiment with 40 combinations each comprising 20 cans. 12 oz. non-lacquered square cans were used.

The present paper concerns 800 cans which comprised the first part of the experiment.

Introduction

Among the products for which CTC (aureomycin) might be used as a preservating agent are luncheon meat. The advantage which might be expected from CTC in this product is prevention of the special off-flavor which often occurs if raw materials (ham trimmings) are stored before the product is made. This is often done in smaller plants. A reduction in that part of can corrosion caused by the bacteriological breakdown of the material might also be expected. Besides this, one might expect CTC to influence the keeping quality of the meat, despite a total destruction of CTC during the heat treatment. Experiments recently completed at the Institute have shown that growth of faecal streptococci in pasteurized

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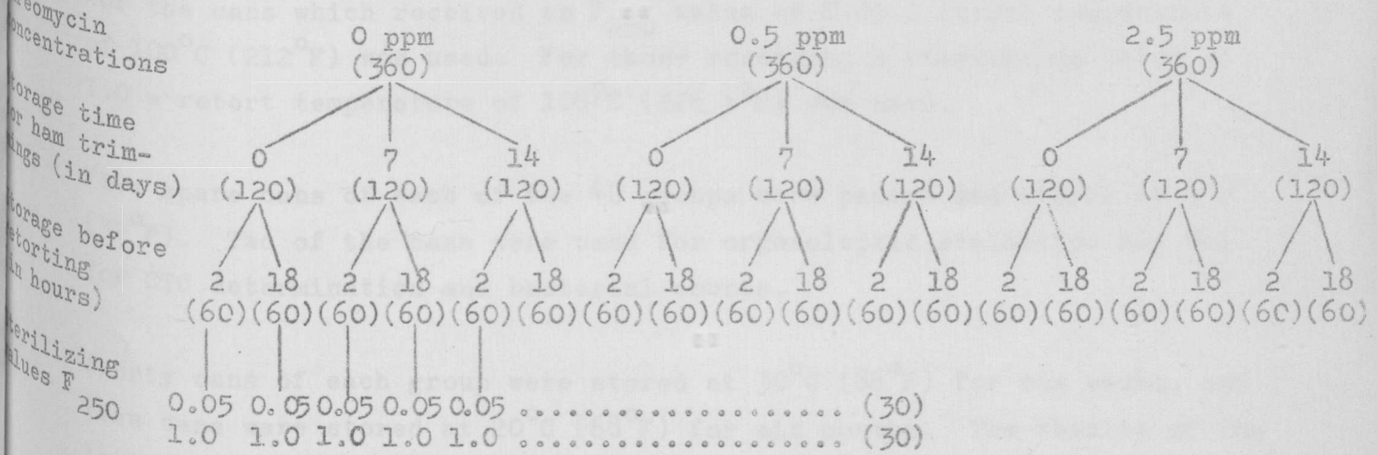
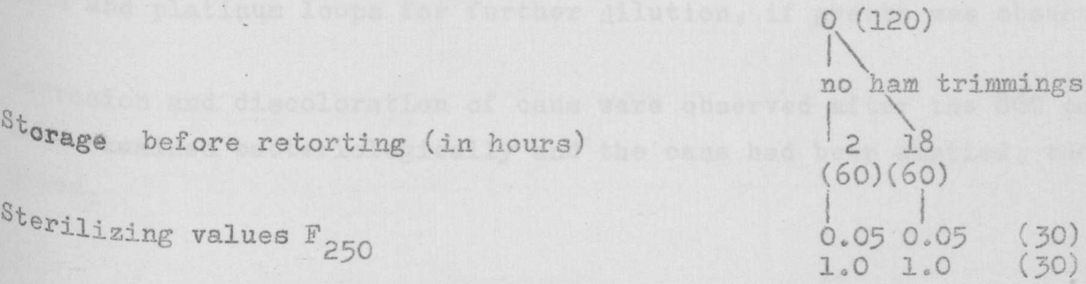
canned hams are inhibited for long periods, 12-14 weeks, after the CTC has disappeared.

The experiment was, as already mentioned, carried out as a full replicate factorial experiment with 40 combinations.

The following plan shows the 36 combinations; the figures in parenthesis indicate the number of cans.



In order to observe the influence of storage of ham trimmings on the characteristics, which were going to be examined, one portion of luncheon meat was prepared without ham trimmings (another 4 combinations). The cans containing that luncheon meat were also kept 2 and 18 hours before retorting. The sterilizing values were the same as already mentioned.



The cans were packed in a meat canning plant and marked with a simple code giving all the necessary information.

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Methods

Fat and lean ham trimmings were kept separately and CTC added to give final concentrations of 0, 0.5 and 2.5 ppm CTC in the finished product. Luncheon meat was prepared from the ham trimmings 0, 7 and 14 days after the beginning of the experiment. CTC determinations and bacterial counts were made on the ham trimmings and the raw luncheon meat.

The cans were packed and stored 2 and 18 hours before retorting.

For the cans which received an F_{250} value of 0.05 a retort temperature of 100°C (212°F) was used. For those receiving a sterilizing value of 1.0 a retort temperature of 108°C (226.4°F) was used.

Five spare cans of each of the 40 groups were packed and stored at 0°C (32°F). Two of the cans were used for organoleptic evaluation and two for CTC determination and bacterial counts.

Twenty cans of each group were stored at 30°C (86°F) for six weeks, and five cans were stored at 20°C (68°F) for six months. The results of the latter are not included in this paper.

The CTC determinations were made according to the method described by Kohler and Abbey (1956). Bacterial counts on raw luncheon meat were made on tomato agar using 4 % saline for dilution as total counts by the drop plate method. The bacterial counts on the retorted luncheon meat were estimated in neo-peptone milk using one gram of luncheon meat as initial load and platinum loops for further dilution, if growth was observed.

Corrosion and discoloration of cans were observed after the 800 cans had been examined bacteriologically and the cans had been emptied, washed and dried.

Registration of the Results

When the experiment was planned it was decided to use punched cards for direct registration of the results since the first part of the experiment

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was to consist of 800 cans and 9 different characteristics were to be registered for each can. Several of the characteristics were evaluated to four or more degrees.

Punched cards for the IBM sorting machine were used. Each card had been coded beforehand with the can code and the number of the can, as each code was present twenty times (see Fig. 1).

When the cans were examined, the data were registered with a pencil according to a pre-fixed code on the punched card corresponding to the can. This code contained the following information: 1) degree of swelling, 2) degree of discoloration, 3) degree of corrosion, 4) degree of deposits from the can on the luncheon meat, 5) scores for smell, 6) scores for the appearance of the surface of the luncheon meat, 7) reaction type in neo-peptone milk, 8) bacterial number, 9) texture of the lunchen meat.

The cards were then sent for punching. One of the advantages of using punched cards was that the statistical treatment of the results was easier. The machine was able to sort the material both into the groups and according to scores in addition to summarizing the material.

Results I

CTC Content in Raw and Retorted Luncheon Meat

CTC determinations in the mixture of ham trimmings gave the following results:

	Storage at 5°C (41°F)		
	after 0 days	after 7 days	after 14 days
Lean ham trimmings having 1.5 ppm CTC added = final concentration 0.5 ppm	0.6 ppm	0.9 ppm	1.2 ppm
Fat ham trimmings having 1.5 ppm CTC added = final concentration 0.5 ppm	0.6 ppm	1.0 ppm	1.2 ppm
Lean ham trimmings having 7.5 ppm CTC added = final concentration 2.5 ppm	3.0 ppm	3.7 ppm	4.7 ppm
Fat ham trimmings having 7.5 ppm CTC added = final concentration 2.5 ppm	2.8 ppm	3.8 ppm	3.4 ppm

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It will be noticed that the findings vary considerably owing to the difficulty often encountered of extracting aureomycin quantitatively from the ham trimmings.

It might, however, be concluded that no demonstrable destruction took place within 14 days at 5°C (41°F).

CTC determination in the raw luncheon meat gave the following results:

	prepared after 0 days	prepared after 7 days	prepared after 14 days
0.5 ppm CTC added	0.1 ppm	0.2 ppm	0.3 ppm
2.5 ppm CTC added	0.7 ppm	0.6 ppm	1.2 ppm

Approximately 40 % CTC was refound in this case.

CTC determinations were also made on one can of each of the combinations after retorting. CTC could not be demonstrated in any of the cases. This meant that less than 0.1 ppm CTC was left in the luncheon meat.

Bacterial Counts of the Raw Luncheon Meat

Total bacterial counts on the raw luncheon meat were made on tomato agar having 2 % glucose added using 4 % saline for dilution. The results are shown in Fig. 2.

It will be noted that even as little as 0.5 ppm CTC in the raw luncheon meat gave lower bacterial counts than the luncheon meat having no added CTC. It was noticed in earlier experiments that as little as 0.5 ppm CTC added to chopped raw ham inhibited the growth of faecal streptococci for a longer period of time.

From the total bacterial counts colonies were chosen by chance and examined morphologically after streaking on milk indicator agar (Richards and Vanderzant (1957)). Fig. 3 gives the percentage composition of the colonies which developed.

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It will be noted that the percentage of yeast increases when the ham trimmings are stored with CTC for 7 and 14 days, whereas rods and cocci seem to decrease or remain at the same level during this period when CTC is added.

Results II

Organoleptic Evaluation

One can of each of the 36 combinations of luncheon meat containing ham trimmings and the cans containing no ham trimmings were evaluated by an expert taste panel. Flavor, texture, saltiness and color were evaluated for all cans. In the following only some of the main effects are mentioned.

When ham trimmings were stored the best scores for color of the luncheon meat were given after 14 days. None of the other treatments had any significant influence on the color of the luncheon meat.

Evaluation of saltiness showed the interesting fact that luncheon meat prepared on the first day of the experiment was judged not salty enough; after 7 days' storage of the ham trimmings the luncheon meat seemed too salty, and after 14 days' storage the luncheon meat was judged to have just the right degree of saltiness. The sterilizing value, too, had en effect on the saltiness, going from 0.05 to 1.0 in sterilizing value gave an increase in saltiness.

The cans containing no ham trimmings were, as already mentioned, also evaluated organoleptically, but the results are not given here. Statistical analysis of the results showed how the addition of ham trimmings contributed to the results. Therefore, the group having no ham trimmings was compared to the group of cans containing luncheon meat prepared with ham trimmings having exactly the same treatments. This meant that the group without ham trimmings was compared to the group prepared on the first day without CTC addition.

No difference could be detected in flavor and color between the two groups. Difference in texture, however, was observed between the two groups.

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Results III

The following characteristics were all evaluated after 6 weeks' storage of the cans in an incubator at 30°C (86°F).

Corrosion of the Cans

When the cans were examined for corrosion, the degree of corrosion was indicated by a scoring system from 1 to 4 . 1 meant no corrosion, 4 very heavy corrosion.

The corrosion was not influenced by the addition of CTC, but although no effect of CTC was observed, this did not mean that CTC had no effect at all on the corrosion. One two-factor interaction was observed where CTC had a significant effect.

Statistical treatment of the material revealed the main effects and interactions of the other treatments given. These results are not given here, but if it is desired to combine the levels so that the best results with regard to corrosion are obtained, one should if CTC is used use

2.5 ppm CTC, store the ham trimmings for 7 days, to give an F_{250} value of 1.0 and a holding time of 18 hours,

or

add 2.5 ppm CTC, store the ham trimmings for 0 days, to give an F_{250} value of 1.0 and a holding time of 2 hours.

Discoloration of Cans

The cans were examined for discoloration, which appeared like black "flames" inside the cans. The degree of discoloration was indicated by a scoring system from 1 to 4. 1 meant no discoloration, 4 heavy discoloration.

The following results apply only to the cans which were allowed to stand for 2 hours.

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When 2.5 ppm CTC was added discoloration was five times as great as when no CTC was added.

The other treatments too effected discoloration. If it is desired to make luncheon meat which gives as little discoloration as possible inside the cans either the closed cans should stand for 18 hours, or if only two hours standing is possible and CTC is used add only 0.5 ppm CTC. The ham trimmings should remain in contact with CTC for 7 days.

Black Deposits on the Surface of the Luncheon Meat

When a can of luncheon meat is opened, small black deposits from the inside of the can may be seen on the surface of the meat. Therefore, the luncheon meat was examined to determine whether these deposits were present.

It showed that addition of CTC as a rule gave more deposits than without CTC.

Some of the other factors, too, had an effect on the amount of black deposits and when ham trimmings were not used in preparing luncheon meat about 30 % more black deposits were observed than when non-stored ham trimmings were used.

If black deposits are to be avoided, only 0.5 ppm CTC should be added and the ham trimmings not stored. An F_{250} value of 0.05 should be included combined with 2 hours' standing of the closed cans before retorting.

Discoloration and Smell of Luncheon Meat.

Any discoloration of the luncheon meat including bleaching of the color and browning was noticed. Addition of CTC in the concentrations employed gave in all cases more discolored cans than when no CTC was added.

Adding 0.5 ppm CTC gave most cans with normal smell, when the ham trimmings were not stored and F_{250} 0.05 was combined with 18 hours' standing or F_{250} 1.0 was combined with 2 hours' standing.

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Texture of Luncheon Meat

The addition of 0.5 ppm CTC resulted in most cans with normal texture. It was also observed that adding 2.5 ppm CTC gave better results than when CTC was not added.

The texture was examined by cutting the block of luncheon meat with a knife and observing whether the texture appeared normal.

Bacterial Counts

As only poor growth was obtained on the agar slants, the bacterial counts were registered only from the neo-peptone medium. When the results were treated statistically, it was observed that the bacterial numbers were spread to a very great extent. This is supposed to be due to the interruption of the growth at different stages of the growth curve, hitting either the lag phase or some place during the logarithmic growth. In order to treat the material statistically it was, therefore, decided to divide the material into commercially sterile and non-sterile cans, the cans with bacterial numbers higher than 10^3 being regarded as commercially non-sterile.

The greatest number of commercially sterile cans were found when 0.5 ppm CTC was added to the luncheon meat. Adding 2.5 ppm CTC gave fewer sterile cans than when no CTC was added. This is remarkable but similar phenomena have often been observed in other experiments with antibiotics. (See Fig. 4)
A rather low concentration of an antibiotic may inhibit growth while a higher concentration may, in a way, encourage growth. This indicates that antibiotics for food preservation can be used only after careful experimentation and not added to products the bacterial population of which is unknown.

The number of commercially sterile cans was influenced, too, by the storage time of the ham trimmings. A storage time of 7 days gave the greatest number of commercially sterile cans.

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The number of commercially sterile cans was not significantly influenced by the F_{250} value given, nor had the standing time any significant main effect on the number of commercially sterile cans. However, the effect of standing time appeared in a significant three-factor interaction.

When luncheon meat prepared without ham trimmings was compared to the normally prepared luncheon meat the following resulted:

The F_{250} value had a significant influence on both sorts of luncheon meat. Luncheon meat with ham trimmings having an F_{250} value of 0.05 and luncheon meat without ham trimmings receiving an F_{250} value of 1.0 gave the greatest number of commercially sterile cans.

Types of Bacteria

The different types of bacteria in neo-peptone milk were noted. Only 3 types were seen: reaction type 0 where no alteration of the milk took place. A growth of this type may be due to certain faecal streptococci or especially to B. licheniformis.

Reaction type 6 appeared as an alkaline reaction of the milk, peptonizing of the milk beginning at the surface, with no gas formation and no putrefactive odour. This reaction type may be due either to B. subtilis or B. vulgatus.

Reaction type 7 appeared as an acidic reaction of the milk, clotting of the milk appearing at the same time. In some cases petonizing was observed. This reaction type may be due either to B. cereus, B. coagulans or B. silvaticus.

In the following the results will be referred to as reaction types in the neo-peptone milk and not to the bacterial species.

Most cans containing bacteria of reaction type 0 were noted when 2.5 ppm CTC was added, 0 and 0.5 ppm CTC giving somewhat fewer cans with type 0 bacteria.

The number of cans containing reaction type O was significantly influenced by the storage time of the ham trimmings. 7 days' storage of the ham trimmings gave the lowest number of cans with type O. Examination of the effect of adding ham trimmings to the luncheon meat seemed to indicate that the addition of ham trimmings increased the number of cans containing type O bacteria.

None of the other treatments had any significant influence on the number of cans containing type O bacteria.

None of the treatments given had any significant effect on the number of cans containing type 6 bacteria.

The number of cans containing type 7 bacteria was influenced by the addition of CTC. The number of cans containing type 7 bacteria increased with increasing amounts of CTC.

Swelling of the Cans

As already mentioned all the above characteristics were evaluated after 6 weeks at 30°C (86°F). After this period no swelling was observed at all.

Conclusion

From the present experiment it may be concluded that the addition of CTC to different products must be done after thorough experimentation. In the case of luncheon meat, 0.5 ppm CTC gave lower bacterial counts than when no CTC was added, whereas 2.5 ppm CTC gave higher bacterial counts than 0.5 ppm CTC. Increasing the dose of CTC thus does not always result in lower bacterial counts. This is, however, only the case after heat treatment of the luncheon meat. Bacterial counts made on the raw luncheon meat indicated that the lowest counts were observed after the addition of 2.5 ppm CTC.

Furthermore, it was observed that addition of CTC increased the growth of yeasts in the raw luncheon meat.

The Possibility of Using CTC in Other Canned Meat Products

Other experiments carried out at the Danish Meat Research Institute have been made on the use of CTC in other canned meat products.

In one experiment the effect of iso-chlortetracycline on faecal streptococci was examined as it had been noticed in earlier experiments that an inhibiting effect was found in heat treated chopped ham even after all CTC had disappeared. This experiment was carried out in nutrient broth by adding different concentrations of iso-CTC. 6 strains of streptococci were used, 4 of them being resistant to CTC. In no case was any inhibitory effect of iso-CTC revealed, which could explain the delay of multiplication of faecal streptococci heated with CTC in such small concentrations that it is all converted to iso-CTC during the heat treatment.

That iso-CTC is not responsible for the delay of multiplication of heated streptococci is also indicated by some preliminary experiments, which showed that CTC may have a considerable effect even when it is diluted away before pasteurization.

The experiment on the effect of CTC in nutrient broth substantiated too the earlier observations with ham, that extremely small concentrations of CTC have a pronounced effect on the sensitive streptococci. Increasing the concentration of CTC does not prolong the lag phase much. The explanation might be that the streptococci absorb a quantity of antibiotic which "saturates" the cell and which is sufficient to prevent multiplication. This "saturation" takes place even when CTC is present in only minute amounts. The streptococci seem to be able to overcome the effect of the absorbed quantity of CTC in 12-14 days under the experimental conditions used, the surplus of CTC being of almost no influence.

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The resistant strains seem to behave in a similar manner although the amount of CTC required for "saturation" of the cells seems higher.

A possible absorption of CTC into the cells explains why treatment with CTC which is eventually diluted away may have a pronounced inhibitory effect. This has been revealed during the experiment mentioned as well as in other experiments, where it was found that dipping hams in CTC dilutions was as effective with regard to the keeping quality of the canned product as injection of CTC with the pump pickle.

According to this, CTC seems to act on faecal streps during ham canning in the following way: small amount of CTC is absorbed into the cells and causes such damage (block) before and possibly during heat treatment that the heated cells recover much more slowly.

From the foregoing it may be concluded that CTC may be used for canned meat products with good results, but that quite a few more experiments have to be done on this subject.

Acknowledgment

The Danish Meat Research Institute wishes to express its gratitude to the American Cyanamid Company for a grant-in-aid to carry out this work.

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377

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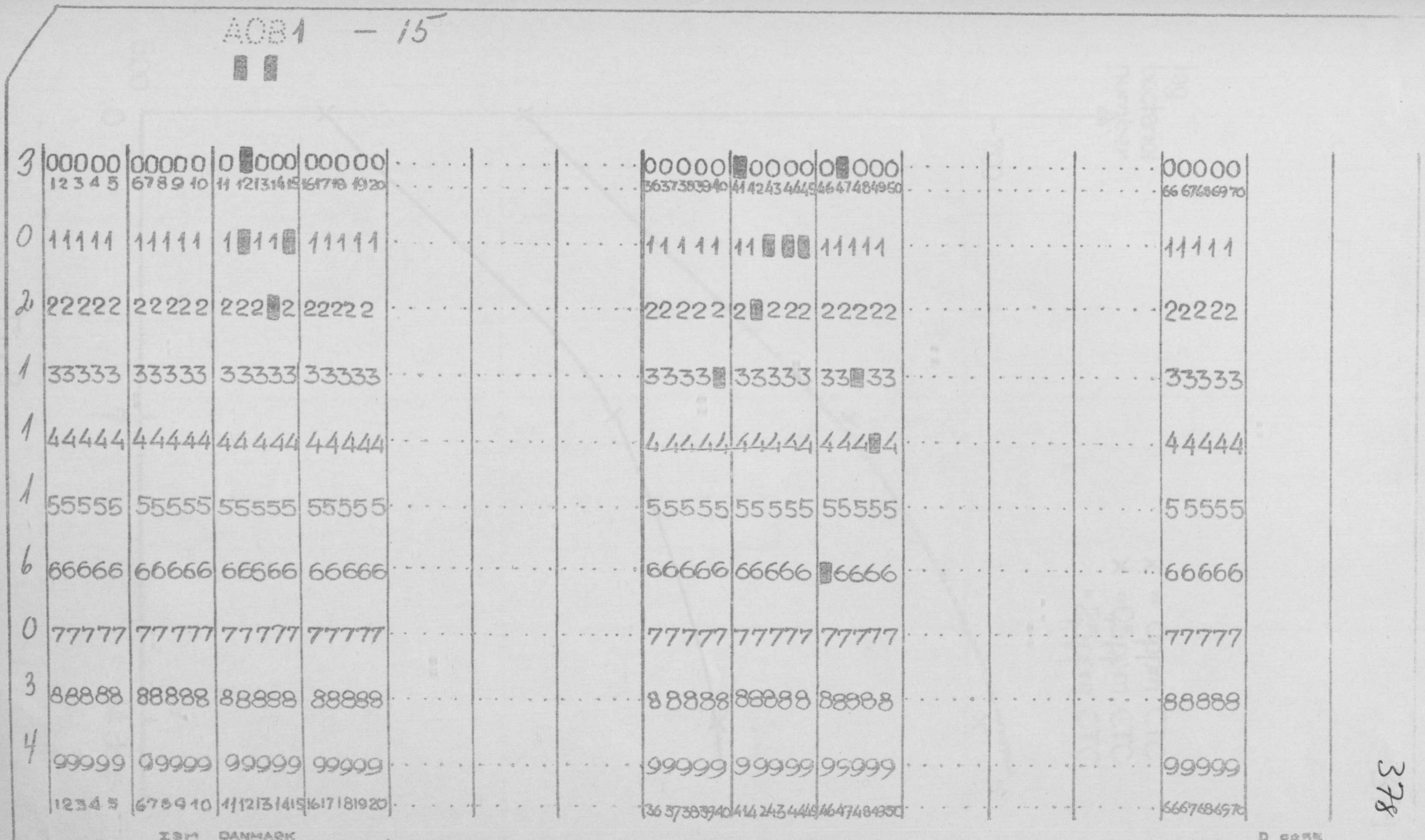

Fig. 1.

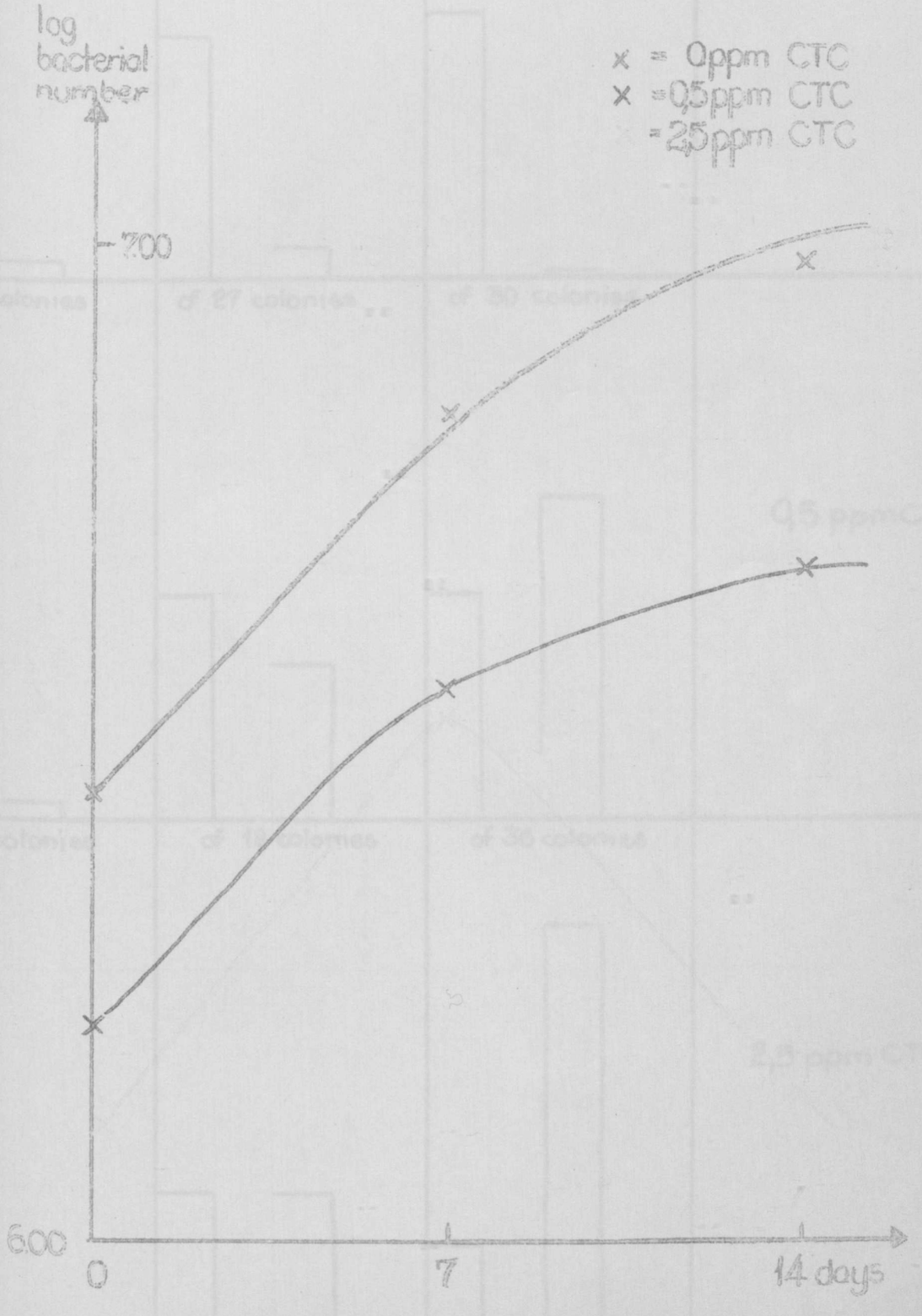
log
bacterial
number
x = 0ppm CTC
x = 0,5ppm CTC
= 2,5ppm CTC
7,00
6,00
0
7
14 days

Fig. 2.

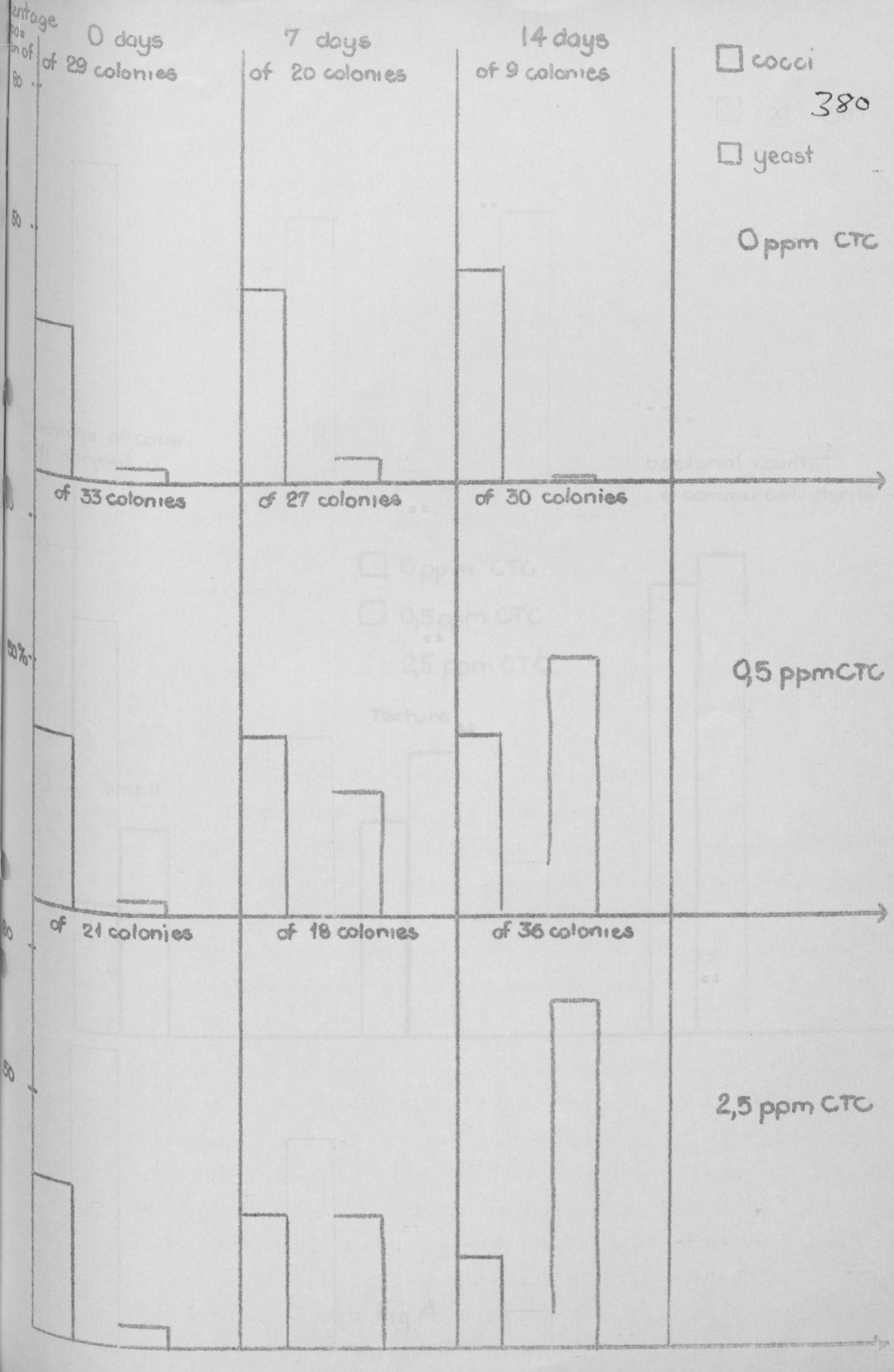
0 days
of 29 colonies
7 days
of 20 colonies
14 days
of 9 colonies
cocci
380
yeast
0 ppm CTC
of 33 colonies
of 27 colonies
of 30 colonies
0,5 ppm CTC
of 21 colonies
of 18 colonies
of 36 colonies
2,5 ppm CTC

Fig. 3

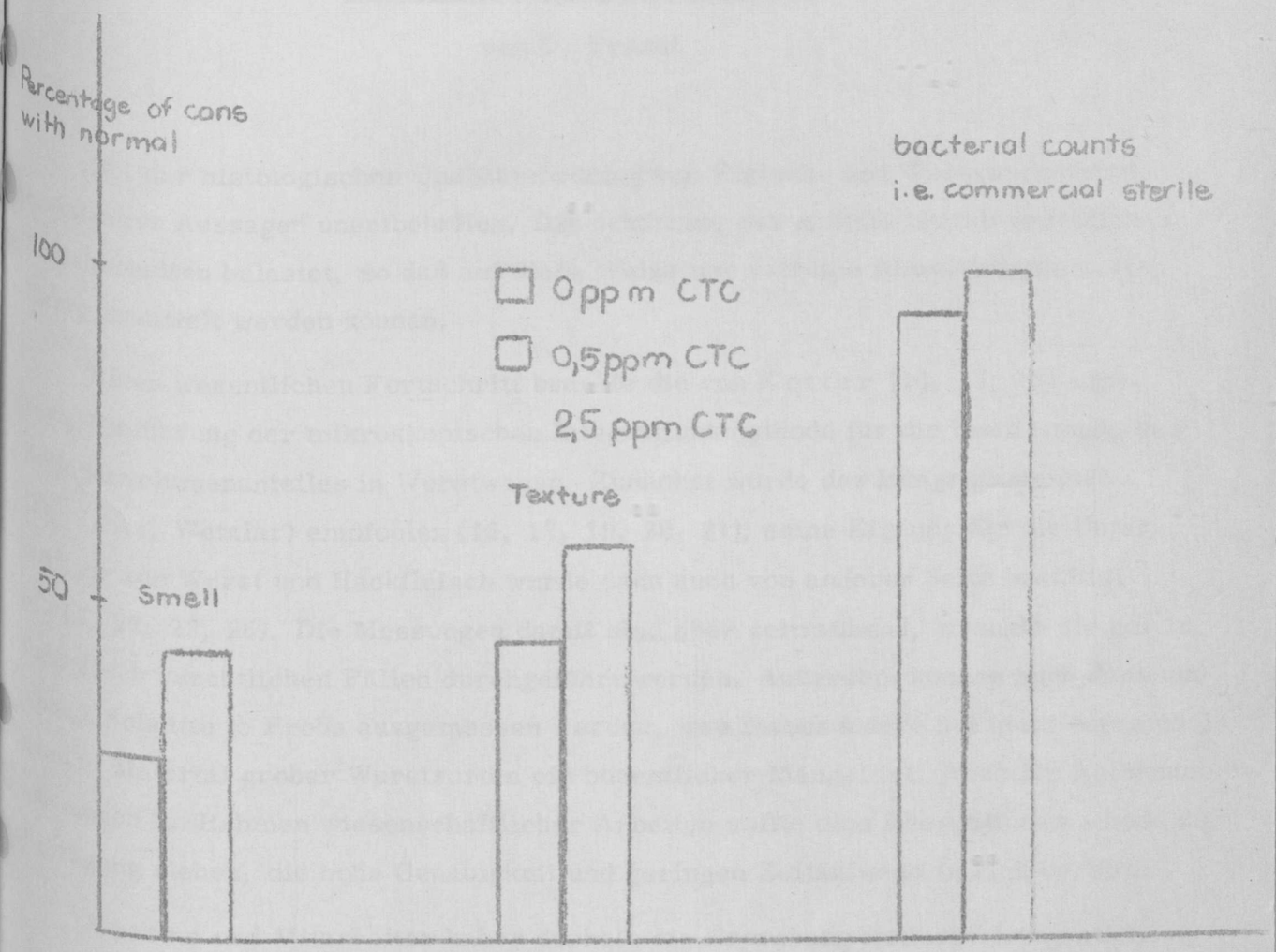
Percentage of cans with normal
100
50
Smell
Texture
0 ppm CTC
0,5 ppm CTC
2,5 ppm CTC
bacterial counts
i.e. commercial sterile

Fig. 4.