Organoleptic Changes and Toxin Production in Canned Meat Experimentally Contaminated with Clostridium botulinum Type B

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Although sanitary and technological regimens have been well established in meat processing industries and beside this the proper knowledge on the potential hazards is widely spread among the country's population, a number of food poisonings are still observed after taking food in form of home - or industrialcanned products /2,3,4/. In Poland, in a relatively - great percentage of food poisonings, as compared with those in other countries, the main causative factor remains C. botulinum /1/.
Botulism is due to the consumption of a product, being in the most observed cases preserved food, in which toxinogenic C. botulinum has been growing on. However, until it is still unknown wheather the sensory organs of a consumer are responsible for the detection of an early multiplication of toxinogenic C. botulinum in food stuffs or that there is some unavoidable margin of such intoxication. This may arise from the predetermined sensitivity of our sensory organs, on the one hand, and from the active and early toxinogenesis, on the other hand. The product of interest may characterize by itself with even small sensory deviations that the consumer ignores them or does not pay any attention to them.
The object of this study was to determine the frequency of appearing the toxin in canned meat product experimentally contaminated with endospores of C. botulinum type B in the period preceding any detectable sensory changes.

Materials and Methods

The following materials were used throughout this study:
1/ endospores of toxinogenic strain of C. botulinum type B No. 366 received from the Veterinary Research Institute, Puławy, Poland. This strain fermented glucose and maltose but did not ferment lactose, saccharose, galactose and salicin. It did not produce indole, digested milk, liquefied gelatin and did not liquefy coagulated serum.
2/ Canned pork minced meat, batch No. 418 A, the production date of April 24th, 1976.
3/ Adult white mice /body weight 18-20 g/ of our own breeding.

Endospores were obtained in Wrzosek's medium by multiplication of the strain in dialysis bags according to the method of Schneider et al. /10/. The endospores were released from bacilli under the action of absolute ethyl alcohol /7/. The density of endospore suspension was determined by serial dilution method. The initial suspension was diluted with saline solution and inoculated into liquid VF medium in a dose of 1 ml /5/. After 24 h incubation period at temperature 37°C the results were read and the highest dilutions determined at which the microorganisms are still growing on. The obtained endospore of density 10^9/ml was frozen in test tubes of 2-ml volume resulting in an uniform initial material for experimental contamination of canned meat products.
In preliminary experiments with canned meat the endospore inoculum and incubation temperature were determined in order to establish the most suitable germination conditions and vegetation periods of C. botulinum in the bulk of a can contents starting from the moment of contamination till the appearance of the first signs of can deformations in a given series of canned meat products or even in a single can. These parameters were determined as a result of a series of can examinations containing the endospore suspension per one can in amounts of 4×10^1, 4×10^2, 4×10^3, 4×10^5, and 4×10^7, respectively. Every endospore dose was added to the series of 30 cans, then afterwards 10 cans were kept at 37°C /the optimum temperature for vegetation/ 30°C /the optimum for toxin production/ /9/ and 17°C /the average temperature in storage rooms/.
Both in the preliminary and in our own present investigations to every canned meat product, a suitable endospore dose was added, in amount of 0.4 ml, by using a syringe and injection through an aseptically mode opening in a can. The opening was then released using the appropriate tin alloy and a soldering gun.
When a first signs of can deformations had been appeared, the whole series of cans were transferred into a storage room /+4°C/. After being sufficiently cooled down the cans were then opened, their contents were examined organoleptically /colour, consistency and smell/ and for presence of botulin toxin. Organoleptic examinations were performed independently by three various examinators. The presence of the toxin was confirmed by the tests made on white mice in accordance to the method of Rymkiewicz /9/.

Results

Table I shows the results of investigations on the effect of inoculum size on the appearance time of the first deformations in canned meat products kept at various temperatures. As optimum value adopted for further investigations the lowest inoculum of 4×10^3 endospores was considered to be adequate at which can deformations were observed. As the optimum temperature 30°C was accepted. At 17°C the germination and vegetation processes were considerably longer and only some portion of the whole number of the examined cans was deformed.

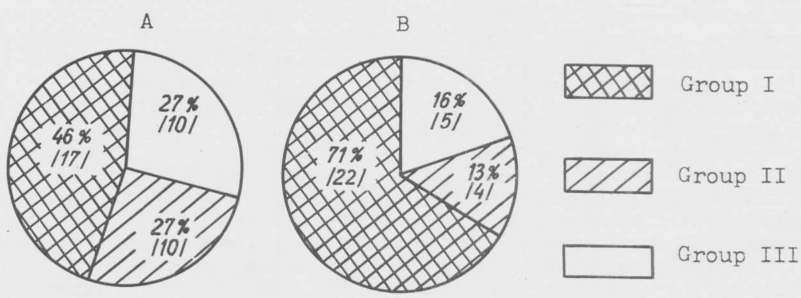
The results of biological test performed with 68 cans of meat contaminated with 4 x 10^3 endospores were shown in Fig. 1. The deformations in that series of cans were noted after 72 hours in 31 cans /B/. In the rest 37 of the cans no deformations were detected even under very carefully performed examination /A/.

Table I. Effect of inoculum size of C. botulinum type B endospores on the appearance time of the first deformations in cans containing meat and kept at various temperatures /the mean values of 10 determinations/

Number of endospores in inoculum	Temperature		
	Appearance time of first deformations in hours		
4 x 10^1	130 ± 36,1	336[1/]	
4 x 10^2	82 ± 11,0	96 ± 0[4/]	
4 x 10^3	72 ± 0	72 ± 0	512 ± 27,7[2/]
4 x 10^5	48 ± 0	72 ± 0	480 ± 0[4/]
4 x 10^7	48 ± 0	72 ± 0	486 ± 12[3/]

Explanations: [1/]No deformations were observed during the period of 336 hours;
[2/]Deformation time of 3 cans;
[3/]Deformation time of 4 cans;
[4/]Deformation time of 5 cans.

Fig. 1. The results of the biological test for the presence of C. botulinum toxin in extracts prepared of contaminated canned meat. A - undeformed cans; B - deformed cans.



Explanations: Group I - the extract lethal for 3 infected mice.
Group II - the extract lethal for 2 or 1 out of 3 infected mice.
Group III - non-lethal extract for 3 infected mice.

All mice that had been given contents of 17 out of 37 undeformed cans died showing the typical symptoms of C. botulinum intoxication /group I/. Apart from this, the contents of 10 other cans caused death of two or at least one out of three intoxicated mice /group II/. In other 10 cans of meat no toxin could be detected in the biological test /group III/. Contents of the deformed cans /Fig. 1 B/ contained the toxin in 22 out of 31 cans /group I/ and the contents from 4 cans were classified into group II due to the ununiformity in mice´s deaths. In the remaining 5 cans of meat no toxin could be detected although multiplication of C. botulinum and can deformations were observed /group III/.
Table II shows the results of the sensory tests reffering to the consumption utility of the contaminated cans being tested at the time of appering the first signs of deformations in the examined series of cans.

Table II. Sensory evaluation of the contents of contaminated cans

Group of cans	Deformed cans: Total number of cans	Number of cans with sensory evaluation: positive[1]	doubtful[2]	negative[3]	Undeformed cans: Total number of cans	Number of cans with sensory evaluation: positive	doubtful	negative
I	8	3	4	1	9	0	5	4
II	10	1	6	3	2	1	0	1
III	8	4	2	2	5	0	1	4

Explanations: 1/Positive sensory evaluation - cans suitable for human consumption /the consistent evaluation issued by three persons/;

2/Doubtful sensory evaluation /the divergent evaluation issued by three persons/;

3/Negative sensory evaluation - cans unsuitable for consumption /the consistent evaluation issued by three persons/.

Discussion

On the basis of the results obtained one might expect that germination of endospores and their vegetation depend to a greater extent rather on the influence of temperature than on the inoculum size. Decreasing the temperature down to 17°C caused a considerable increase in time needed for deformations to be observed. Similar results were obtained. Similar results were obtained by Skoczek /11/ in his studies on the influence of physical and chemical agents on germination and growth of C. botulinum type E in canned fish.
Riemann /8/ has found that under unfavorable conditions a greater number of endospores is required for the toxin production than for C. botulinum type E vegetation in contaminated food. These Riemann's findings could be used for explanation the fact of endospore germination and C. botulinum vegetation in our own studies which finally has lead to can deformation although the results of the biological test for the presence of the toxin in the contaminated canned meat were negative.
The positive results obtained in the biological test indicate to endospore germinations and the toxin release prior to can deformations and sensory changes could be detected. In the available literature there are data concerning the relationship between sensory changes observed and the toxin production. Only Hojer et al. /6/ were able to detect the botulinum toxin in organoleptically unchanged ham sausage slicers incubated under vacuum conditions. In our present studies we were able to detect the toxin in nearly half of the whole number of undeformed cans. Futhermore, a great majority of these cans gave no rise to any objections as to the organoleptic tests performed by three persons taking part in their examination. For a half of them, they issued the divergent estimation and only for a smaller portion of them their evaluation was consistent as to their unsuitability of the canned meat human consumption.
On the basis of the results obtained it is possible to draw a conclusion that under closely predetermined circumstances the process of the toxin production may precede gas accumulation arising from metabolic processes in bacilli that are responsible for the appearance of can deformations as well as for arising other sensory changes detectable by a potential consumer. If it is assumed as unavoidable the random character of the presence of C. botulinum in raw materials and in final food products then we must recognize with the risk of sensory deficiency in a consumer of such products in which early but toxinogenic vegetation has taken place. This phenomenon may explain why there are still observable cases of C. botulinum intoxication caused by canned food. The trend to limit this risk should be involved in further increasing sanitary conditions in food processing industry, namely in improving the sanitary and hygienic status of raw materials.

Conclusions

1. The deformation of canned meat contaminated with endospores of C. botulinum type B depends to a greater degree on the temperature of bacterial multiplication than on inoculum size.
2. Toxin production may precede can deformations and arising any sensory changes in canned food contents.

References

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