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STERILISATION OF CANNED MEATS WITH REGARD TO THE RESISTANCE OF BAC. STEAROTHERMOPHILUS SPORES. - R. Žakula

The studies on the thermal resistance of Bac. stearothermophilus have a great theoretical and practical significance in determination of sterilisation in the canning industry. In the majority of the published papers, the resistance of this organism has been examined from the aspect of fruit and vegetables preservation, and the troubles arising due to flat sour changes in this type of canned products (4,5).

In the available literature there are no data on the changes caused by Bac. stearothermophilus in canned meats. On the other hand, by organoleptic examination of canned meats to which the starch had been added (chopped pork, corned beef), we had opportunities to find the souring of the contents without signs of blowing. Also, in many samples of starch and sugar, taken from the stores of meat industry, this organism was isolated.

These findings were direct reason for studies on the thermal resistance of Bac. stearothermophilus spores, suspended in water solutions of some curing ingredients (salt, nitrates, nitrites), in the water solution of curing ingredients mixed in definite relation, as well as in emulsions of chopped beef and pork.

Materials and methods.

For the thermal resistance studies was used the strain of Bac. stearothermophilus CP 2325, isolated from a can of chopped pork. After several inoculations on the dextrose triptone agar, the spores were prepared for further studies.

To obtain the spores, the strain of Bac. stearothermophilus was inoculated on agar with 30 ppm $MnSO_4.H_2O$ and 0,05% glucose. This medium was spilt in a thick layer in Petri plates of a greater diameter. The medium was then incubated for 6 days on 55°C, after which it was held for 5 days on the room temperature for autolisis of the vegetative forms. The grown up colonies were washed away from the agar with the neutral phosphate buffer. The obtained suspension of spores was then centrifugated 5 times on 3.000 revolutions per minute with the pouring off the supernatant liquid. The spores suspended in the phosphate buffer were kept for further use in refrigerator on the temperature of 4°C. The relation of spores and vegetative forms in the suspension was determined microscopically and there were more than 95% spores. For that reason, the suspension was not heated for the inactivation of vegetative forms before the examination of the thermal resistance. The count of spores in 1 ml of the suspension was 10^7.

Thermal resistance of spores of the strain of Bac. stearothermophilus CP 2325 was examined in the following substrates:

- distilled water,
- water solution of NaCl (3 percent),
- water solution of $NaNo_3$ (o,2 percent),
- water solution of $NaNO_2$ (0,015 percent),
- water solution of 3% NaCl + 0,2% $NaNO_3$ + 0,015% $NaNO_2$
- emulsion of chopped pork, and
- emulsion of chopped beef.

In the experiment on the thermal resistance, the method of capilary tubes was used. The glass capillary tubes with thin wall had the inside diameter of 3 mm, and the length of 80 mm. For each heating period 10 capillary tubes were used.

Into each previously sterilized capillary tube, open on both ends, under aseptic conditions and with sterile syringe with long and thin injecting needle, was inserted 0,3 ml of spore suspension in the studied liquid substrate. To fill the capillary tubes with emulsion

of beef or pork, a filler was constructed the cylindar of which can receive about 50 g of chopped meat. The piston of the filler moves over a microscrew and on the fore end is fixed an injecting needle with the outside diameter of 2,5 mm, and the length of 100 mm. After filling with substrate melting on both ends, the capillary tubes were placed in the metal stands and heated in oil bath, the temperature of which was controlled with precision of $\pm$ 0,1°C. The capillary tubes were heated to the temperature of: 100, 105, 110, 115 and 121°C. The come-up-time for the capillary tubes was found by measuring with the thermocouple, and it was: 21 second for 100° 20 seconds for 105°, 19 seconds for 110°, 18 seconds for 115°, and 16 seconds for 121°C. After heating, the capillary tubes were taken out of the oil bath and placed into ice-water the temperature of which was under 5°C. The rinsing of the cooled capillary tubes was carried out, first in 96% alcohol and then in sterile distilled water. After rinsing each capillary tube was placed into a porcelan friction cone, previously filled with 10 ml of sterile neutral phosphate buffer. The capillary tubes were then crushed by a pestle and after prolonged mixing, 1 ml was taken and inoculated into melted dextrose triptone agar. The Petri plates were then incu bated for 45 hours on 55°C. For each examined temperature and substrate a spore surviving curve was constructed on the semilogarithmic paper by plotting the time of heating on the abscissa and log number of survivors on the ordinate. To obtain z values, on the ordinate were plotted D values and on the absciss the temperatures on which the experiment on thermal resistance was carried out.

Results and Discussion

The results obtained in experiments on thermal resistance of Bac. stearothermophilus CP 2325 in different substrates and on different temperatures, are shown in tables 1,2 and 3. As materials for those three tables were used the graphs made on the semilogarithmic paper. They were not enclosed to this paper due to their great number (42).

In the table 1, where are shown the F values for Bac. stearo-thermophilus spores suspended in different substrates and treated by different temperatures, it is shown that these values are inversely proportional to the temperature. By their effects on the decrease of F values on all the examined temperatures, the water solution of $NaNO_3$ and $NaNO_2$ are particularly outstanding. In the other group, with almost the same but remarkably lesser effect on F values are distilled water, water solution of sodium chloride and water solution of curing ingredients mixed in the definite relation. Only on 121°C all curing ingredients individually or in mixture have an equal affect on F values. As it is shown in the table 1, beef and pork differ by their effect on F values from curing ingredients. In pork till the temperature of 121°C always a higher F value was obtained and only on the highest examined temperature the same F value in both meats was obtained.

In the table 2 are shown D values obtained in the experiments on thermal resistance of Bac. stearothermophilus CP 2325 in different substrates. As it could be expected, D values decreased with the increase of temperature. There are data in the literature that D value does not depend on the initial bacterial concentration. (10,15) although there are some reverse data (1,11). On the ground of our study, we are more inclined to accept the standpoint that D values depend on the initial bacterial concentration, because D values are defined from the organisms surviving curve, and the slope of this curve will depend on the initial concentration of microorganisms in the examined substrate.

It is interesting to emphasize the fact that in all the examined substrates an agreement between D and F values was noticed. On the ground of those observations, Schmidt's suggestion (15) on convertibility of D into F values and in reverse might be accepted, by the formula:

$$F = D\,(\log A + 2) \qquad \text{or} \qquad D = \frac{F}{\log A + 2}$$

On converting one value into another, there arise certain differences between values obtained experimentally and the computed values, but they are neglegible.

In ourwork relatively small differences in z values were observed in respect to the substrate in which were suspended the Bac. stearophermophilus spores (table 3). However, the highest z values were obtained in water solutions of $NaNO_3$ and $NaNO_2$ (18^oF), and the lowest in the pork emulsion ($15,5^oF$). On the ground of our results on obtained z values, a conclusion that the suspending substrate has a definite effect on the z values can be drawn.

Before further continuing the discission on our results, it should be emphasized that, in respect to the conditions of cultivation in our experiments, we had spores from rough colonies of Bac. stearothermophilus, which are by Fields (3) less resistant than the spores originating from smooth colonies.

Here, by all means, a fact should be emphasized, and that is that F values for Bac. stearothermophilus spores suspended in distilled water, 3% NaCl and mixture of curing ingredients in a definite relation, used in the commercial practice, were very similar. As there are no data from other authors on the effect of curing ingredients on Bac. stearothermophilus, some comparisons to other organisms will be made. Riemann (1963) has examined the effect of heat treatment and curing components on the pA 3679 spores. The examined factors in this experiment were: NaCl, $NaNO_3$, $NaNO_2$, pH and F_o value. The mutual reactions were statistically significant on the 5% and 1% level of probability: F_o x NaCl, F_o x $NaNO_2$; NaCl x $NaNO_3$ x $NaNO_2$; NaCl x pH and NaCl x $NaNO_3$. The observation in this work prove that the preservation system of cured meat is complex and that one factor cannot be evaluated without taking the others into account.

Sugar and curing components in the laboratory nutritive media have a very mild effect on the thermal resistance of putrefactive anaerobic spores. Gross et al. (8) have found that the

decimal reduction time on 250°F for the spores of the putrefactive anaerobe S_2 is of the order 1,1 - 1,2 minutes. The same authors on the ground of other data (7) pointed out that a certain smaller number of spores has a higher resistance that the one expressed from the decimal reduction time for the majority of spores.

Rogatchyov, Mazokhina and Bogdanova (14) have examined certain regularities following the destruction of Bac. aerothermophilus spores on 110 - 150°C in neutral phosphate buffer. This obligate thermophile with high resistance causes a change in the pH value of the substrate without gas forming, and by its properties is very close to Bac. stearothermophilus. It was mentioned in this work that the surviving curve can be divided into three parts: 1. the initial period that shows a lag of spores destruction, 2. the destruction period of the basic mass of spores, which shows a logarithmic dependence of the number of spores on the heating time, and 3. the period of prolonged destruction of a small number of spores, which can be twice or more times longer than the time needed for the destruction of the basic mass of spores in the first two periods.

From a great number of data it shows that the spores naturally present in meat, have remarkably lower resistance than that of the spores PA 3679 and the spores S_2, produced in laboratory conditions. By the data from Gross (6) the decimal reduction time of the naturally present putrefactive anaerobic spores is approximately 0,3 minutes on 250°F. Riemann (12) has found that the spores of PA 3679 inoculated in chopped cured ham, have the decimal reduction time on 250°F of the order 0,6 minutes. The spores of Cl botulinum A and B in neutral phosphate buffer, have the decimal reduction time of the order 0,3 - 0,4 on 250°F (2,9).

Conclusions

On the ground of the data obtained in this work on the values for the decimal reduction for the Bac. stearothermophilus spores

on 250F, which are of the order 0,14 - 0,17 minutes, it may be concluded that the spores of this organism, produced under laboratory conditions, poses a relatively low thermal resistance in the presence of curing ingredients in relation to the spores PA 3679 and the spores of Cl. botulinum A and B.

Thus, the organoleptic changes in canned meats, which are characteristic for the biochemical activity of Bac. stearothermophilus, show that in the commercial practice sometimes very mild sterilisation procedure is applied, which lead to undesirable outcome and deterioration of canned meats.

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Table 3

z values of Bac.stearothermophilus spores suspended in different substrates./

z values in °F.						
Distilled water	3% NaCl	0,2 % $NaNO_3$	0,015% $NaNO_2$	3% NaCl 0,2% $NaNO_3$ 0,015% $NaNO_2$	Beef	Pork
16,0	16,0	18,0	18,0	17,0	16,0	15,5

B7

D values of Bac.stearothermophilus spores at various temperatures suspended in different substrates.

Table 2.

Temperature °F	Temperature °C	D values in minutes: Distilled water	3% NaCl	0,2% $NaNO_3$	0,015% $NaNO_2$	3% NaCl 0,2% $NaNO_3$ 0,015% $NaNO_2$	Beef	Pork
212	100	4,1	4,0	2,1	2,1	4,0	4,7	5,0
221	105	2,4	2,4	1,2	1,2	3,1	3,0	3,4
230	110	1,1	1,1	0,5	0,4	1,1	1,4	1,8
239	115	0,8	0,5	0,2	0,1	1,0	1,1	1,4
250	121	0,14	0,14	0,14	0,14	0,14	0,17	0,17

B7

F values of Lac. stearothermophilus spores at various temperatures suspended in different substrates

Table 1

Temperature °F	Temperature °C	F values in minutes: Distilled water	3% NaCl	0,2% $NaNO_3$	0,015% $NaNO_2$	3% NaCl 0,2% $NaNO_3$ 0,015% $NaNO_2$	Beef	Pork
212	100	29,0	28,0	15,0	15,0	24,0	33,0	35,0
221	105	17,0	17,0	9,0	9,0	19,0	21,0	24,0
230	110	9,0	8,0	4,0	3,0	7,0	10,0	12,0
239	115	6,0	4,0	2,0	1,0	6,0	8,0	10,0
250	121	0,5	0,5	0,5	0,5	0,5	1,0	1,0

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