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Contribution to the Study of the Thermoresistance of Bacillus Spores Suspended in Different Substrates.

If numerous factors upon which the canned meats sterility depends, although the commercial one, are considered, one can be convinced that the industrial practice, which does not pay an adequate attention to these factors, is often faced with some unpleasant surprises related to the poor keeping quality of a greater or smaller number of the productive lots of cans.

Technical and scientific literature is scanty with data on the thermoresistance of micrococci, streptococci, bacilli and clostridia in comminuted beef and pork in the presence of the curing ingredients. Especially, few data are available on the values required for estimating the sterilization and pasteurization of canned meats. Without those data, derived from the thermal death time curves, survivor curves and thermal destruction curves, determination of the exact process times could not be considered that would provide bacteriologically valid cans.

Keeping quality of canned meats is mostly affected by the microorganisms from Bacillus and Clostridium genera as in most cases their spores are very thermoresistant. Meat mass prepared to be canned, depending upon the meat contamination and general hygienic conditions of the production, contains a higher or smaller count of bacilli spores that have to be destroyed applying the adequate thermal treatment.

Certainly, in addition to heat, some other factors influence thermoresistance of the spores, e.g., curing ingredients in the cans produced of the cured meat.

In the present work, which is a part of the complex problem on the thermoresistance of microorganisms and estimation of the sterilization and pasteurization of the canned meat, that is under the study, determination of the F, D, z_F and z_D values as the indices of the thermoresistance for the following bacillus species: Bac. licheniformis, Bac. subtilis, Bac. cereus, Bac. megatherium and Bac. polymixa is performed.

Spores of the bacilli studied were suspended in the following substrates: the neutral phosphate buffer, the water solution of the curing ingredients, the comminuted beef with and with-out curing ingredients added and the comminuted pork with and without curing ingredients added.

Materials and method

All bacilli were isolated from the changed and unchanged meat cans. To obtain the spores, the medium of the following composition was used: 2% agar, 30 ppm $MnSO_4.H_2O$ and 0,05 % glucose. The colonies grown in the Roux flasks were washed with the neutral phosphate buffer and water solution of the curing ingredients, respectively. After homogenization, several repeated centrifuging and followed with removal of the supernatant, the spore suspension was obtained and by the microscopic examination it was found to contain about 95 % of spores. The spore suspensions prepared were stored in the electric refrigerator at the temperature of 3°C until use, but no longer than two weeks. The neutral phosphate buffer had pH 7,0 and the water solution of the curing ingredients contained 3% NaCl, 0,2 % $NaNO_3$ and 0,015 % $NaNO_2$. Larger pieces of beef and pork were free of fatty and connective tissue,

burnt from all sides on the burner, comminuted in the sterilized meat chopper and finally, meat was homogenized in the mixer with 30 % of the saline.

To one portion of the homogenized meat the curing ingredients were added in the relationship equivalent to that in the water solution.

In all substrates examined the concentration of spores amounted to 2 to 3 x 10^7.

The thermoresistance of the above mentioned bacilli was tested by the capillary method (length 120 mm and i.d. 3 mm). After it is filled with substrates (0,3 ml fluid and 0,3 g meat), the capillaries were melted at both ends on the burner. In the oil bath with contact thermometer, accuracy $\pm$ 0,1°C, the capillaries were treated at the temperatures of 100, 110, 115 and 121°C for the period ranging from 15 seconds to 130 minutes.

From the results obtained the thermal death time curves, survivor curves and thermal destruction curves are plotted on the semi-log paper and from those curves the F, D, z_F and z_D values are determined.

Results and discussion

The fundamental task of this study was to investigate the effect of the curing ingredients on the thermoresistance of the selected bacilli species. As regards the above, the basic conclusion is that the curing ingredients in the concentrations used, increase the thermoresistance of all five studied bacilli. In the water solution of the brine ingredients, the thermoresistance was higher as compared to the neutral phosphate buffer, it was higher in the beef and pork with the curing ingredients added as compared to the meat without those ingredients added.

Out of five bacilli species studied, the highest thermoresistance was found in Bac. licheniformis in all sub-

strates used. It is worth mentioning that the greatest protective power was exhibited by the pork with the curing ingredients added. In this substrate, the F_{212} is for five minutes higher in comparison with the pork without the curing ingredients added. In the beef this difference was smaller and amounted only 3 minutes for the F_{212}. At higher temperatures these differences decreased, thus, the F_{250} amounted to 1,3 and 1,2 in pork with and without the curing ingredients added, respectivelly. In beef, at that temperature, the difference was greater and the F_{250} was 0,5 and 1,1 with and without the curing ingredients added, respectivelly. Almost the same relation were maintained in the case of the D values. Bac. licheniformis had the highest z_F and z_D values in comparison with the other bacilli tested.

Considering the F, D, z_F and z_D values obtained for the other studied bacilli it could be concluded that their thermoresistance, on the basis of those indices, is very similar and considerably lower than that of Bac. licheniformis. Anyhow, in this group, Bac. subtilis and Bac. cereus have slightly higher thermoresistance as compared to the others.

The other workers as well as the authors in the previous papers have found that Bac. licheniformis species has higher thermoresistance than the other species from the same genus. The rise of the thermoresistance of bacilli in the presence of the curing ingredients could be explained by the fact that the salt, in the concentration we have used (3 %), increases the thermoresistance of spores while the other curing ingredients in the presence of a higher count of the spores (in out case 10^7) do not show any inhibitory activity. Pork has a better protective power as compared to beef, maybe, owing to its higher fat content (pork 3 %, beef 1,5 %) or, that seems more likely, due to the different chemical composition of fat

of these two kinds of meat. The above is under study.

Conclusion

On the basis of the results obtained the following conclusion can be drawn:

- Out of five bacilli species studied the highest thermoresistance in the presence of the curing ingredients in all substrates was exhibited by the spores of Bac. licheniformis,
- lower but rather good thermoresistance of the others was found in Bac. subtilis and Bac. cereus spores,
- according to the protective power, the substrates can be classified in the following manner: pork with the curing ingredient added > pork without the curing ingredients > beef with the curing ingredients added > beef without curing ingredients > the water solution of the curing ingredients > the neutral phosphate buffer,
- in the complexity of the factors in meat affecting the bacterial thermoresistance, certainly, fat has its determined role,
- a better protective power of pork, in our opinion, could be explained rather on the basis of the difference in the chemical composition of pork and beef fat than on the basis of the difference in the percentage, within our studies; in order to elucidate these phenomena the experiments are undertaken that are in the course of the study.

Comparative F, D, z_F and z_D values for five Bacillus species

Table 1

Bacillus species	Temperature °F	Temperature °C	Neutral phosph. buffer F	Neutral phosph. buffer D	Water solution of cur.ingred. F	Water solution of cur.ingred. D	Beef without cur.ingred. F	Beef without cur.ingred. D	Beef with cur.ingred. F	Beef with cur.ingred. D	Pork without cur.ingred. F	Pork without cur.ingred. D	Pork with cur.ingred. F	Pork with cur.ingred. D
Bac.liche-niformis	212	100	110	17,0	112,5	17,5	115	10,0	118	18,0	120	18,0	125	17,5
	221	110	30,5	4,0	32,5	4,0	35	3,7	35	6	45	5,0	40	4,5
	230	115	7,5	0,9	8,0	0,9	11	0,98	12	2,0	14,8	1,8	13	1,5
	250	121	0,45	0,06	0,52	0,06	0,72	0,07	1,1	0,18	1,2	0,18	1,1	0,12
			z_F 17	z_D 17,0	z_F 18	z_D 17,5	z_F 18	z_D 18	z_F 18	z_D 18	z_F 18	z_D 18	z_F 17,5	z_D 17,5
Bac.subti-lis	212	100	105	16	109,5	17,0	108	16,0	110	16,0	112	17,5	116	17,5
	221	110	28	3,3	31,5	4,5	32	4,0	50	4,5	41	3,5	42	3,8
	230	115	5,5	0,7	7,0	1,1	8,1	0,9	15	1,2	13	0,8	14	0,9
	250	121	0,23	0,03	0,42	0,07	0,50	0,04	0,8	0,08	0,9	0,04	0,9	0,04
			z_F 15	z_D 15	z_F 17	z_D 17,5	z_F 16	z_D 15	z_F 16	z_D 17	z_F 17,5	z_D 15	z_F 17,5	z_D 15
Bac.ce-reus	212	100	103	15,5	107	15,5	104	15,5	108	16,5	110	16,5	112	16,5
	221	110	25	3,8	30	3,5	30	3,5	38	3,8	40	3,5	41	4,0
	230	115	4,5	0,8	6	0,75	7,5	0,82	10	1,4	11	0,85	11,5	0,9
	250	121	0,13	0,045	0,31	0,037	0,38	0,04	0,65	0,8	0,7	0,04	0,7	0,04
			z_F 14	z_D 16,5	z_F 16	z_D 15	z_F 15,5	z_D 15	z_F 16,5	z_D 16,5	z_F 16,5	z_D 15	z_F 16,5	z_D 15
Bac.mega-terium	212	100	100	15	103	13,5	100	17,5	105	17,0	107	16,5	108	17,0
	221	110	21	3,5	25	3,0	35	3,0	32	4,5	38	3,5	36	3,7
	230	115	3,3	0,65	4,2	0,7	10	0,7	9,5	1,5	10,2	0,8	10	0,85
	250	121	0,1	0,03	0,17	0,037	0,70	0,03	0,6	0,1	0,65	0,04	0,7	0,04
			z_F 14	z_D 15	z_F 15	z_D 16	z_F 17,5	z_D 15	z_F 17	z_D 17,5	z_F 16,5	z_D 15	z_F 17	z_D 15,5
Bac.poly-mixa	212	100	101	16	105	16	104	17,5	106	16,5	108	17,0	110	17,0
	221	110	23	4,0	28	3,3	33	3,5	38	4,2	38	3,8	38	4,0
	230	115	3,5	0,7	5	0,6	9,8	0,8	9,5	1,3	10	0,95	11	0,9
	250	121	0,12	0,04	0,25	0,027	0,70	0,04	0,60	0,12	6,5	0,5	0,7	0,05
			z_F 14	z_D 15	z_F 15	z_D 14	z_F 17,5	z_D 15	z_F 16,5	z_D 18	z_F 17	z_D 15	z_F 17	z_D 16