Results on investigations of thermoresistence of some bacteria suspended in meat, lard and tallow

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This report is aimed to represent some ot our results we have obtained in long time investigation on the complex problem of thermoresistence of bacteria suspended in different substrates. In literature data are available on thermal resistence of some species of microorganisms but, however, data are still lacking concerning the influence of salt, nitrates, nitrites and polyphosphates on thermoresistence and particularly scarce on the effect of lard and beef tallow. According to our past investigations pork and beef, respectively, make two completely different environments relative to the influence on thermoresistence of bacteria.

MATERIAL AND METHODS

The bacterial strains, isolated from meat cans and semicans of meat were used in our studies. Out of 15 isolated bacterial strains, for further investigations we only used those showing the greatest thermoresistence in the neutral phosphate buffer and water solution of the brine ingredients (3 % NaCl, 0.2 % $NaNO_3$ and 0.015 % $NaNO_2$) and they were B. licheniformis, M. candidus and Str. faecalis.

The suspensions of the known concentration were prepared from tested bacterial strains, which were added to different substrates. To obtain B. licheniformis spores the suspensios were heated to 80° C for 15 minutes, repeatedly centrifuged and the supernatants were decanted each time.

Pork and beef were prepared in the following way: the surface area of the piece of meat was cleaned thoroughly from adipose and connective tissues, burnt from all sides and coagulated external surface layer was removed with the sterile knife. Pieces of meat were comminuted in the sterile mixer with 10 % of physiological solution until meat mass of pastelike consistency resulted. The whole meat mass was contaminated with the suspensions of bacterial strains so that there were 2 to 3 $\times$ 10^7 bacteria in one gramme of meat. Then the meat mass was halved and the brine ingredients (3 % NaCl,

0.2 % $NaNO_3$ and 0.015 % $NaNO_2$) were added to one part. Pork had pH 6.0 and 3 % of fat and beef pH 5.9 and 1.5 % fat.

To study the activity of polyphosphates on thermal resistence of bacteria, meat mass was prepared as above, and the polyphosphates »Polital M5A», »Regal Brine Mix» and »Tari P II» were added to meat in the form of water solution to reach the concentration of 0.4 %.

Pork lard and beef tallow were melted to the temperature slightly above the melting point, then they were added to the test tubes in which the bacteria were precipitated by centrifuging and the supernatant liquor was decanted. In fats the bacteria concentration, i.e., spores amounted to 10^7. Lard and beef tallow were taken immediately after melting of adipose tissue. Lard contained 0.2 % of moisture and tallow 0.25 %.

In our studies on thermoresistence of bacteria, the capillary method was used in all substrates. The glass thin wall capillaries had 3 mm in internal diametre and they were 8 cm long. Prior to filling of the capillaries by the filling device, of our own design, the capillaries were open at both ends. After the capillaries had been filled with the substrate, at the amount of 0.2 g, they were sealed at the both ends in the flame and kept in iced water until the beginning of heating. For any time and heating temperature, we used 10 capillaries. For the temperatures below 100° C we used the water bath and the temperatures above that value the oil bath. After the termination of the treatment time, the capillaries were plunged into iced water at once, then in alcohol and finally in sterile distilled water. The capillaries were crushed with the sterile forceps in the centrifuge tube with 3 ml of physiological solution and 1 ml of this substrate was subcultured on the nutritive culture media. To detect B. licheniformis and M. candidus survived we used nutritive agar and for Str. faecalis the nutritive culture medium with sodium azide with 3 % of defiberized sheep blood added.

The results on the thermal treatment of the studied bacterial strains, suspended in the above mentioned substrates, are presented in the table as the values of D.

RESULTS AND DISCUSSION

Influence of NaCl and the other brine ingredients on thermal resistence of spores in the process of sterilization of canned meat has been reported in the works of Yesair and Cameron (1942), Stumbo *et al.* (1945), Bulman and Ayres (1952), Jensen (1954), Gross (1954), Riemann (1963) and others. Most cited authors reported that NaCl and the other brine ingredients (sodium nitrate and sodium nitrite) in the concentrations, normally found in the cured meat, do not considerably affect the length of the sterilization process needed for the cans of cured meat. By Silliker (1958) inhibitory activity of the brine ingredients is not displayed if the meat contains a great number

of spores. By Riemann (1963) 90 per cent or more of species from the genus Bacillus, isolated from canned meat, could multiply in the presence of 10 % of salt. Jensen (1954) reported that NaCl exerted protective activity against micrococci in the course of the thermal treatment. Niinivaara (1955) stated that nitrites at the highest concentrations (0.002 %) did not have any effect on micrococci.

On the basis of our results, presented in table 1, the conclusion could be drawn that thermoresistence of the studied bacterial strains, suspended in comminuted pork and beef with the brine ingredients added, was slightly increased.

From these experiments it could be also concluded that pork had better protective power compared to beef to all bacterial strains studied. In literature we used in the interpretation of our observations and results this fact is not so clearly emphasized.

In the continuance of our investigations we studied the influence of polyphosphate preparations on thermoresistence of B. licheniformis spores. In literature, data treating this problem, are very scarce. Brachfeld (1955) studied the activity of phosphate buffer on thermal resistence of spores and observed that only M/20 phosphate buffer considerably decreased the number of the survived while M/80 to M/2.000 did not exert any influence on germination and multiplication. Williams and Hennessee (1956) came to the similar conclusion as they observed that by decreasing the molar phosphate concentrations in the range of M/15 to M/120, resistence of spores was increased to the lethal temperature (120° C). Ordal and Lechowich (1958) studied the effect of phosphates on resistence of spores and abserved that they reduced their resistence independently to the concentration. Finley and Fields (1962) explained the effect of phosphates in the combination with heat suggesting that phosphate ions interfere the complex mineral — dipicolinic acid in the spores on which thermoresistence of spores depended.

Kelch and Bühlmann (1958) studied the effect of polyphosphates »Curafos» and »Fibrisol» on the growth of some species of bacteria (M. aureus, Str. faecalis, B. subtilis, Cl. sporogenes and Cl. bifermentans). Discussing their observations, these authors indicated that »Curafos» had the stronger bacteriostatic activity than »Fibrisol». Ana Oluški (1963) studied thermoresistence of M. aurantiacus, M. candidus and M. agilis in the broth without polyphosphate and with 0.5 % of polyphosphate »Tari» added. In the conclusion she stated that thermoresistence of tested micrococci was smaller in the broth with the polyphosphate preparation added in all cases.

The results from the above cited works are hardly comparable with our results because different polyphosphates, different microorganisms and different substrates for making suspensions were used.

In our investigations we used the following polyphosphate preparations:

»Polital $M5_A$» — Yugoslav product, »Regal Brine» — American product and »Tari P II» — West German product, at the concentrations as used in large-scale industrial production.

Besides the fact that in this work we used the physiological solution, water solution of the brine ingredients, pork and beef as the culture media, in this report we would only briefly summerize the results obtained with pork and beef to which three kinds of polyphosphates were added.

When B. licheniformis spores were suspended in the comminuted pork and three, above mentioned, polyphosphate preparations were added to that substrate, at lower testing temperatures (100 and 110° C) slightly lower values of D were recorded in comparison with the meat without polyphosphate. At the temperature of 121° C no differences were observed and the same values of D were recorded ($D_{121} = 0.2$) in the meat with and without polyphosphate added.

Spores of B. licheniformis, suspended in beef with the brine ingredients, showed twice as smaller thermoresistence ($D_{121} = 0.08$) compared to pork. Addition of polyphosphates to beef meat had no influence on resistence of B. licheniformis spores in that substrate.

According to the results obtained in this investigation, the conclusion could be drawn that polyphosphates in the substrates from pork and beef in the presence of the brine ingredients, at higher sterilization temperatures, do not affect thermal resistence of B. licheniformis spores. This statement can be important for large scale industrial sterilization practice of canned meat.

Within the complex of the factors affecting thermoresistence when suspended in the meat substrate, fat certainly plays a defined role, certainly. Stumbo (1965), in his book, reported that higher concentrations of fat in meat, under determined conditions, increased thermoresistence of bacteria. Lang (1935) suggested that fat had poorer heat conductivity. According to the studies of Sigiyama (1951) thermoresistence of Cl. botulinum spores was increased in the presence of long chain fatty acids. If water was present in fat, resistence of bacteria was considerably reduced, probably, because the coefficient of convection of such a mixture increased. Rogachewa (1940) conducted the corresponding tests with the bacteria of the group Subtilis — Mesentericus and confirmed the above presented statement. Jensen (1954) studied thermoresistence of streptococci, suspended in moist fat, and reached the same results. Namely, streptococci in moist butter endured the temperature of 100° C only for 15 minutes and in the dry one 50 minutes at 115° C or 20 minutes at 120° C. Molin and Snygg (1967) found that thermoresistence of tested bacilli was considerably increased when palmitic acid was added to soybean oil.

In one of our works we studied thermoresistence of M. candidus, Str. faecalis and spores of B. licheniformis, suspended in pork lard and beef tal-

Table 1a. *Comparative D values for B. licheniformis spores, M. candidus and Str. faecalis*

Substrate	*Bacterial strain*								
	B licheniformis			*M. candidus*			*Str. faecalis*		
	100	*110*	*121*	*60*	*70*	*80*	*60*	*70*	*80° C*
Pork without curing ingredients	44,0	4,5	0,35	9,0	1,0	0,11	6,5	0,5	0,04
Pork with curing ingredients	50,0	5,7	0,43	10,0	1,3	0,14	8,0	0,65	0,06
Beef without curing ingredients	22,5	2,2	0,09	7,5	0,8	0,07	3,1	0,3	0,02
Beef with curing ingredients	25,5	2,3	0,1	8,3	0,7	0,06	3,2	0,23	0,02
Pork with curing ingr. + Polital	42,0	12,0	0,2	—	—	—	—	—	—
Pork with curing ingr. + Regal	44,0	10,0	0,2	—	—	—	—	—	—
Pork with curing ingr. + Tari	42,0	11,0	0,2	—	—	—	—	—	—
Beef with curing ingr. + Polital	22,0	5,0	0,09	—	—	—	—	—	—
Beef with curing ingr. + Regal	25,7	5,6	0,08	—	—	—	—	—	—
Beef with curing ingr. + Tari	21,4	5,0	0,08	—	—	—	—	—	—
Lard	73,4	6,9	0,4	32,0	2,9	0,25	43,0	3,5	0,28
Tallow	35,8	3,3	0,2	15,7	1,35	0,09	20,0	1,6	0,13
Pork with curing ingr. + Polital + 10 % lard	—	—	—	—	—	—	8,5	0,6	0,54
Pork with curing ingr. + Polital + 20 % lard	—	—	—	—	—	—	9,5	0,78	0,06
Pork with curing ingr. + Polital + 30 % lard	—	—	—	—	—	—	10,5	0,92	0,08
Pork with curing ingr. + Polital + 40 % lard	—	—	—	—	—	—	10,8	1,1	0,13

Table 1b. *Comparative D values for B. licheniformis spores, M. candidus and Str. faecalis*

Substrate	*Bacterial strain*								
	B. licheniformis			*M. cancidus*			*Str. faecalis*		
	100	*110*	*121*	*60*	*70*	*80*	*60*	*70*	*80 C*
Beef with curing ingr. + Polital + 10 % tallow	—	—	—	—	—	—	7,5	0,75	0,06
Beef with curing ingr. + Polital + 20 % tallow	—	—	—	—	—	—	8,0	0,8	0,08
Beef with curing ingr. + Polital + 30 % tallow	—	—	—	—	—	—	9,0	0,95	0,1
Beef with curing ingr. + Polital + 40 % tallor	—	—	—	—	—	—	11,0	1,1	0,12

low at the temperatures of 60, 70 and 80° C, and 100, 110 and 121° C, respectively. Lard was obtained by the moist procedure in the De Laval equipment and beef tallow by melting in the open cauldron. Lard contained 0.2 % of moisture and beef tallow 0.25 %.

The values of D obtained for three species of bacteria, studied inthis work, showed that lard considerably increased resistence of these microorganisms to heat effect. For example, D_{121} for B. licheniformis spores was 0.4 minute while D_{100} 73.4 minutes. M. candidus had $D_{80} = 0.25$ minute and $D_{60} = 32.0$ minutes. Thermoresistence of Str. faecalis in this substrate was even higher and D_{80} amounted to 0.28 and D_{60} 43.0 minutes.

Protective activity of beef tallow on three studied bacterial species compared to that of lard was twice or more times smaller. For example, B. licheniformis, suspended in lard was killed at 121° C within 4 minutes while suspended in beef tallow it was killed after 1.1 minutes. M. candidus in the lard was killed at 70° C for the period of 20 minutes and in beef tallow it lost its vital characteristics after 9 minutes. Str. faecalis was killed at 60° C within 300 minutes in the lard and after 140 minutes in beef tallow.

In the studies on the protective effect of varying percentages of lard and beef tallow added, respectively (table 1), the conclusion could be stated that by increasing the percentage of fat added the values of D were increased. In these experiments it was noticed that in the substrates from pork, the values of D were higher in the pork compared to the values in the beef at all tested temperatures.

How fat protects bacteria against the heat effect, namely lard better and beef tallow poorer, the conclusion is still uncertain. The differences in the chemical composition and physical characteristics, certainly, play a significant role. Under the determined conditions, free fatty acids become »electrostatically activated» and they easily associate forming double bond hydrogen bridges. Association of free fatty acid molecules effects the increase in the molecular weight and, evidently, in the boiling point. Perhaps, the raised boiling point and coating of bacteria with the fatty acid associates, in addition to the factors, have an effect on the increase of thermoresistence of microorganisms, suspended in fat or substrates with a higher percentage of fat.

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