Inhibitory Effect of Pickle Salts on Sublethally Heated Spores of Bacillus in Dependence on Initial Number of Bacteria and pH Substrate

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Interduction

Spores that survive heat influence during sterilization of meat cans remain in the state of slowed down or inhibited metabolic activity. This state is known under names: dormany, slowed down germination, latent life, abiose, cryptobiose (5,1o,11,14,16,2o,24) in references. It is cosidered to be the result of sublethal effect of heat (4,6,8,17,19,21,22). The survived spores are damaged by heat to the degree that, unless they are exposed to specially suitable conditions, they are not able to express their biology activity. Inactivity of survived spores is considerably contributed by environment factors / pickle composition, pH, temperature, etc./ (7). The cans containing live, in usual keeping coditions inactive, bacteria or spores, are called "comercially sterile", which means practically stable spores (1, 2,3,8,13).

The factors supporting inertia of survived spores in the meat cans are numerous, their influence being complex and still insufficiently investigated. The pickle composition / sodium chloride, nitrite, nitrate / has special significance, either individually or in interaction with pH substrate.

Former research of influence of sodium chloride, nitrate, nitrite and some other mixtures, as well as pH in the meat cans concerning prevention of development of spores damaged by sublethal temperatures, significantly contributed to the comprehension of the problem. Nevertheless all aspects of the problem are still insufficiently investigated (9.17,21,23,25).

In favour of recognition of the above mentioned problem, we tried to determine "in vitro" the effect of inhibitory influence of sodium chloride, nitrite, nitrate and pH substrate, individually and in interaction, onto bacillus spores treated by sublethal heat in dependence of their initial number.

Material and method

The spore culture of the bacterium type form Bacillus family, being isolated from the meat cans were cultivated by Kim and al. (12) method. It was decided to use diluted suspension of spores which, after thermal treatment was applied, gave cca loo and looo colonies/ml, respectively, on Tryptosa agar base with starch.

As base were used Tryptose infusion and Tryptose agar with starch, pH 6,o and 7,o (3) with addition of various concentrations of pickle composition. Three nutritious bases were inoculated for each test and results are worked out as medium values.

The number of spores which survived test heat treatments was determined by method

Table 1. Microbial and shelflife characteristics of beef tongues and livers prior to and following transcontinental and transoceanic shipment.

Characteristic	Tongues			Livers		
	Processing plant prior to freezing	Following transcontinental shipping	Following transoceanic shipping	Processing plant prior to freezing	Following transcontinental shipping	Following transoceanic shipping
35° APC[a]	c	2.56[d]	1.52[e]	c	3.44[d]	2.71[e]
20° APC[a]	3.10[d]	2.82[e]	2.67[e]	3.15[d]	3.15[d]	2.88[d]
7° APC[a]	c	2.30[d]	2.10[d]	c	2.43[d]	1.98[d]
Light brown color[b]	16	3	0	0	0	0
Moderate reddish brown color[b]	0	31	98	28	18	0
Vivid red color[b]	27	3	0	0	0	0
Dark purplish red color[b]	0	17	0	4	3	0
Dark red[b]	0	0	0	17	4	21
Blackish purple[b]	0	0	0	0	21	0

[a]APC=Aerobic plate count, counts = $\log_{10}/cm^2$

[b]Frequency to the nearest percent of all colors scored for either livers or tongues at that particular phase of shipping. Not all colors assigned are given.

[c]Counts not determined due to limited facilities.

[d,e]Means in the same row within tongues or livers bearing different superscripts are significantly different ($P<0.05$).

Table 2. Microbial and shelflife characteristics of beef tongues and livers before and after specified handling and storage conditions.

Variety meat, Characteristic	Initial	Packaging, handling and storage conditions					
		Abused			Control		
		Vacuum packaged	Film wrapped	Naked	Vacuum packaged	Film wrapped	Naked
Tongues							
20° C APC[a]	3.61[d]	4.08[d]	6.03[c]	5.82[c]	3.38[d]	3.54[d]	3.59[d]
Anaerobic bacteria	3.65[c,d]	3.34[d]	3.87[c,d]	4.46[c]	2.85[d]	2.59[d]	3.15[d]
Yeasts and molds	1.17[d]	0.89[d]	2.22[c]	2.69[c]	1.24[d]	1.06[d]	1.25[d]
Moderate reddish brown color[b]	24.1	33.3	61.0	50.0	15.4	23.1	27.3
Vivid red color[b]	39.2	5.6	0.0	0.0	7.7	7.7	9.1
Moderate red color[b]	5.1	22.2	4.9	6.5	23.1	23.1	36.4
Dark reddish gray color[b]	0.0	0.0	19.5	17.4	15.4	23.1	0.0
Very dark red color[b]	0.0	18.5	2.4	2.2	15.4	0.0	27.3

of the most probable number in infusion with starch (3) and results were interpreted in accorance with tables (28).

Sodium chloride, nitrites and pH were determined by standard methods (26,27).

Results and Discussions

Test results are shown on tables 1.- and 2.-.

The following conclusions can be drawn out of them:

Sodium chloride in tested concentrations from 1,5 to 3,5 % has inhibitory effect on development of B. subtilis spore previously exposed to temperatures from 8o and 9o°C during the period of 3o minutes / table 1.- /. The effect of inhibitory influence increases together with increase in concentration of NaCl, reduction of initial number of spores and decrease in pH. In the most suitable proportion of those factors, the number of developed colonies was smaller for 88,9%, comparing with control base.

Bacteriostatic effect of sodium nitrite and nitrate varies in dependence on concentration, initial number of spores and pH base. Expressed by the number of grown out colonies in comparison with control culture, it ranged from 6,2 to 88,9 %, i.e. from 3,8 to 77,8%.

Inhibitory effect of sodium chloride and nitrite mixture, expressed in the same way, was from 25,o to 1oo,o % / table 2.- /, and that of sodium chloride and nitrate was from 8,7 to 88,9 %. The highest inhibitory effect had the mixture of all tested ingredients / NaCl, $NaNO_2$ and $NaNO_3$ /.

Table 1.- Inhibitory effect of NaCl, $NaNO_2$ and $NaNO_3$ in different conc. on B. subtilis spores exposed to t of 8o and 9o°C for 3o min in dependence on initial number and pH base

number of spores/ml after heating	% of ingredient in base		spore heating temperature / °C /			
			8o		9o	
			pH base			
			7,o	6,o	7,o	6,o
			percentage of grown out colonies			
875	NaCl/g	1,5	94,9	92,4	9o,8	87,7
		2,5	88,o	67,6	64,4	49,9
		3,5	77,4	66,1	52,6	35,3
8o		1,5	56,2	43,7	38,8	33,3
		2,5	37,5	25,o	22,2	16,6
		3,5	31,2	18,7	16,6	11,1
875	$NaNO_2$/mg	o,53	93,8	91,7	85,9	81,1
		3,12	84,9	75,4	62,8	51,6
		5,25	76,1	63,9	5o,7	34,8
8o		o,53	43,7	31,2	27,7	22,2
		3,12	31,2	25,o	22,2	16,6
		5,25	25,o	18,7	16,6	11,1
875	$NaNO_3$/mg	o,78	96,2	95,1	94,o	93,1
		5,97	94,9	91,8	85,7	78,9
		8,46	9o,9	86,3	82,4	75,6
8o		o,78	61,7	5o,o	44,4	38,8
		5,97	47,2	37,5	33,3	27,7
		8,46	43,7	31,2	27,7	22,2

Table 2.- Inhibitory effest of mixtures of NaCl, $NaNO_2$, and $NaNO_3$ on B. subtilis spores exposed to t emperature of 8o and 9o°C for 5o min in dependence on initial number and pH base

number of spores/ml after heating	% of ingredient in base	spore heating temperature / °C / 8o pH base 7,o	8o 6,o	9o 7,o	9o 6,o
		percentage of grown out colonies			
875 8o	NaCl 2,5% and $NaNO_2$ 5,31 mg%	75,o 16,6	61,6 11,1	44,o 11,1	25,5 0,o
875 8o	NaCl 2,5% and $NaNO_3$ 8,7 mg%	91,3 27,7	81,4 16,6	63,9 16,6	55,9 11,1
875 8o	NaCl 2,5% and $NaNO_2$ 5,3 mg% $NaNO_3$ 8,7 mg%	74,9 16,6	64,8 11,1	45,8 11,1	29,3 0,o

In relation to the tested bacteria types of Bacillus family, essential differences were not noticed in respect of inhibitory effect of above mentioned factors / it is somewhat less expressed in relation to the type B.cereus /. The influence of temperature of heating and initial number of spores, the effect of inhibition with all tested types is noticable. Growing of colonies from culture treated at loo°C was for 2,7 /B.cereus/, 3,4 /B.subtilis/ and 4,8 /B.licheniformis/ times less if compared with cultures tested at 8o°C and at spores initial number les for 11,1 /B.subtilis and B. licheniformis/, and 17,6 times /B.cereus/, respectively.

Increasing of pH substrate increases inhibitory effect of pickle ingredients. The same effect could be also expected in the content of meat cans as they belong to the group of mild acid food / pH around 6,o /.

Conclusions

NaCl, $NaNO_2$ and $NaNO_3$ used individually and in a mixture, in permitted concentrations, have inhibitory effect on bacillus spores previously exposed to sublethal heat. The effect of inhibitory influence is more strongly expressed in a mixture with a more intense thermal treatment of spores, less initial number and lower pH substrate.

NaCl, $NaNO_2$ and $NaNO_3$ used in a mixture, show stronger inhibitory effect than each of them used individually.

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