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EFFECT OF pH AND BRINE CONCENTRATION ON THE THERMAL RESISTANCE OF PA 3679 IN A PROCESSED CHEESE SPREAD [1, 2]

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SUMMARY

Tests conducted on a processed cheese spread inoculated with PA (putrefactive anaerobe) 3679 and packaged in TDT (thermal death time) cans indicated that only a small amount of heat would prevent gas production as measured by can expansion, whereas a considerably greater amount of heat would kill the spores. The average D_{250} values of PA 3679 in cheese spread at pH 5.50, 6.25, and 7.00 were 0.67, 1.01, and 1.21 min., respectively, with a z value of 18° F.

The results suggest that a surviving spore population in the range of 10^3 to 10^5 per gram is necessary for gas production by this organism in the processed cheese spread tested. As the processing temperature increased, the calculated number of spores necessary to initiate gas production decreased.

The thermal resistance of a spore suspension of PA 3679 in neutral phosphate buffer was equivalent to a D_{250} value of 0.98 with a z value of 17.5° F., as measured with the thermoresistometer technique and subsequent subculturing in liver infusion broth.

Heat treatment may be used to render processed cheese spreads virtually free of spoilage organisms without undue damage to the product (9, 10). However, under commercial processing procedures there have been instances of gas production attributable to spore-forming anaerobes in the cheese. The objective of this study was to determine the thermal resistance of PA (putrefactive anaerobe) 3679 in a processed cheese spread under varying conditions of pH and brine concentration. The problem was studied in two parts: (a) the effect of pH and brine concentration on the thermal resistance of the spoilage organism; and (b) the effect of pH and brine concentration on the heat treatment required to prevent gas production by the spoilage organism.

EXPERIMENTAL PROCEDURE

The thermal resistance of a spore suspension of PA 3679 prepared by Jaynes *et al.* (7) was determined using a thermoresistometer which has negligible heating and cooling lags (11). Heat resistance was evaluated at temperatures of 240, 245, 250, 255, and 260° F. At each of these temperatures ten replicate

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samples were tested at eight to ten different time intervals. After the heat treatment the samples were incubated at 98.6° F. in stratified tubes of liver infusion broth (7). Both positive and negative growth occurred in the series of tubes in at least two of the time-temperature combinations tested at each of the above-designated temperatures, enabling use of the Schmidt (14) method to evaluate the data.

All tests to determine the effect of pH and brine concentration on the thermal resistance and the heat treatment required to prevent gas production of PA 3679 were made in TDT (thermal death time) cans (208 × 006). The TDT cans were filled with 19 g. of inoculated standardized processed cheese spread substrate prepared by Jaynes *et al.* (7). The substrate contained an amount of spore suspension calculated to give 3.3×10^6 spores per can or 1.74×10^5 spores per gram, salt to give the desired brine concentration, and either 6.0 *N* NaOH or HCl as necessary to give the desired pH. The TDT cans of inoculated cheese spread were allowed to equilibrate at 56° F. before being heated in a flat position in the miniature retorts. All heating times were corrected for heating and cooling lags (8).

Tests to determine thermal resistance. Thermal resistance studies of PA 3679 were made on inoculated batches of standardized processed cheese spread at pH levels of 5.50, 6.25, and 7.00 and at brine concentrations of 2.0 and 4.4% for each of the three pH levels. Processing temperatures of 225, 230, 235, 240, and 245° F. were used. TDT cans of cheese prepared and processed as described above were incubated overnight at 98.6° F., then approximately 0.01 g. of product from each can was removed with a loop and subcultured in stratified liver infusion broth. The 0.01-g. sample was obtained by stabbing the central area of the aseptically opened TDT can of product in three different places with a calibrated loop. After the third stab, the loop of product was introduced into the stratified tube of subculture medium. All tubes were incubated at least 3 mo. at 98.6° F. Five replicate TDT cans were used at each time-temperature combination and one tube was subcultured from each can. The remaining portion of cheese was subjectively evaluated for color. The D values were calculated for the data of each temperature and the F values of the contents of the TDT cans were calculated from the D values, using the methods of Schmidt (14).

Tests to determine heat treatment required to prevent gas production. Studies on the gas production by PA 3679, as evidenced by expansion of TDT cans, were made on batches of standardized cheese at pH levels of 6.0 and 7.0, brine concentrations of 1.6 and 2.4%, and processing temperatures of 220, 225, 230, and 235° F. The thickness of each of ten replicate TDT cans incubated at 98.6° F. at each time, temperature, pH, and salt combination were measured, using a dial micrometer technique, previously described by Jaynes *et al.* (7). The cans were measured daily for the first three days after heat processing, then monthly for 5 mo. All measurements for thickness of cans were made at a constant temperature of 98.6° F. Results in terms of positive can expansion and no can expansion were plotted on semilogarithmic paper and a straight line drawn above all positive values.

RESULTS AND DISCUSSION

Effect of pH and brine concentration on thermal resistance. The thermal resistance of the spore suspension of PA 3679 heated in M/15 phosphate buffer and subcultured in liver infusion broth was normal for this organism under these conditions; the D_{250} was 0.98 min. and the z was 17.5° F.

Results of the test to determine the thermal resistance of PA 3679 in cheese spread at pH levels of 5.50, 6.25, and 7.0, with brine concentrations of 2.0 and 4.4%, are presented in Table 1 and shown graphically in Figure 1. F values were calculated using Schmidt's (14) equation:

$$F = D \ (\log \ A + 2)$$

Since the initial number of organisms (A) in the can was 3.3×10^6, then $F = 8.52 \ D$. The D_{235} values decreased with reductions in the pH, but were not affected by variations in brine concentration. The thermal resistance of

TABLE 1

Effect of pH and brine concentration on D_{235}, F_{235}, and z values for TDT cans of cheese spread containing 3.3×10^6 spores of PA 3679 per can (0.01 g. from each can subcultured in liver broth and incubated at 98.6° F. for 3 mo. or more)

pH	Brine concentration (per cent)					
	2.0			4.4		
	D_{235} (min.)	F_{235} (min.)	z (° F.)	D_{235} (min.)	F_{235} (min.)	z (° F.)
5.50	4.6	39.2	18.25	4.5	38.3	19.25
6.25	6.8	58.0	18.75	7.0	59.6	18.25
7.00	8.4	71.6	17.75	8.1	69.0	17.00

the spores is significantly greater at pH 7.0 and 6.25 than at pH 5.5, as determined from the 95% confidence limits. Differences in the thermal resistance of the spores between pH 6.25 and 7.0 are not statistically significant. The decrease in the thermal resistance of the spores at reduced pH values observed in this work verified the findings of other investigators (2-4, 13, 15).

Thermal resistance curves vary with the methodology used in obtaining them. The error involved in removing 0.01 g. of sample from each TDT can for subculturing is estimated to be about plus or minus 20%. Since logarithms are used in the calculations, there would be practically no change in the calculated D values unless the subcultured samples were either at least ten times greater or ten times smaller than 0.01 g. In addition to the error in sample weight, there are inherent errors attributable to nonuniform distribution of the spores and to determination of the most probable numbers.

Failure of variations in brine concentration to affect the thermal resistance of the spores substantiated the results of Yesair and Cameron (18), who reported that the thermal resistance of spores of *Clostridium botulinum* was unaffected by different concentrations of salt when the samples were subcultured in an optimum growth medium. Salt did not show any protective influence in this study, which is contrary to results reported by some other workers (5, 16, 17).

The relative position of the color curve and the F curves in Figure 1 indi-

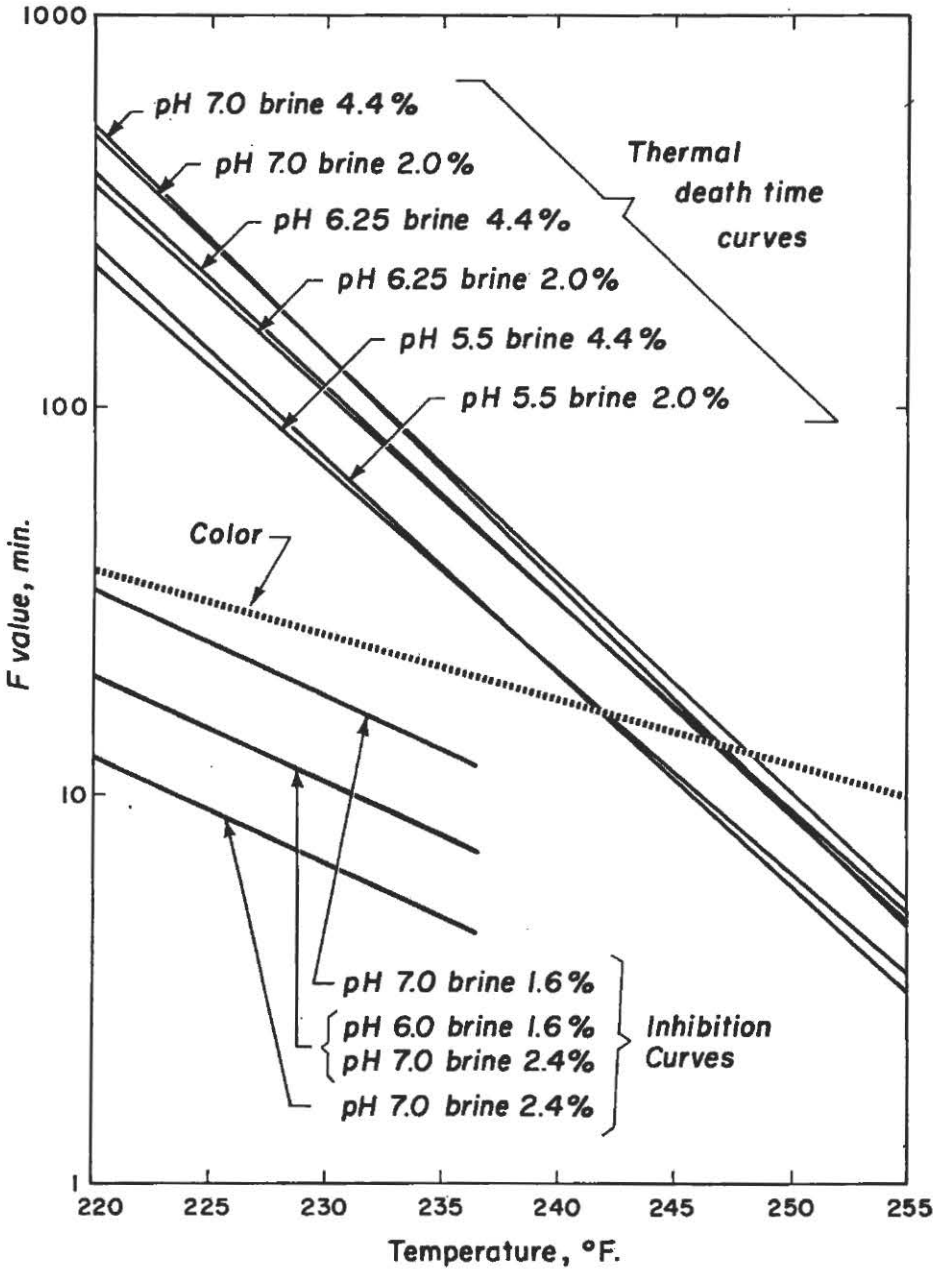

FIG. 1. Graphical summary showing thermal death time, inhibition, and color curves.

cate that thermal processes at higher temperatures for shorter times are preferable to low-temperature long-time processes for producing a commercially sterile product with an acceptable color. Under conditions of this study, minimum temperatures that could be used to commercially sterilize TDT cans

(208 × 006) of processed cheese spread at pH 5.50, 6.25, and 7.00, and maintain an acceptable color, were 242, 247, and 248° F., respectively. This points out the necessity for using high temperatures for short times to obtain a commercially sterile processed cheese spread with an acceptable color. These conclusions agree with Ball (1), who pointed out that, in proportion to their destructive effect on bacteria, high temperatures have less effect on quality impairment than low temperatures. Under conditions of these tests, an F_{250} of approximately 10 min. was required for commercial sterility at a pH of 6.25 or 7.0.

Effect of pH, brine concentration, and the thermal process on the number of spores necessary to cause expansion of cans. Data presented in Table 2 and

TABLE 2

Effect of heat treatment (U) at different retort temperatures on the expansion of TDT-cans of cheese spread at several pH and brine concentrations (3.3×10^6 spores of PA 3679 per can and incubated at 98.6° F. for 5 mo.)

Retort temperature							
220° F.		225° F.		230° F.		235° F.	
U (min.)	Expansion (in.)	U (min.)	Expansion (in.)	U (min.)	Expansion (in.)	U (min.)	Expansion (in.)
		pH 6.0 and a brine concentration of 1.6%					
7.6	0.112	5.4	0.100	4.0	0.115	2.9	0.107
12.5	0.095	9.0	0.089	6.6	0.094	4.8	0.098
20.0	0.022	14.5	0.025	10.5	0.051	7.8	0.057
33.0	0.000	24.0	0.000	18.0	0.000	13.0	0.000
		pH 6.0 and a brine concentration of 2.4%					
4.6	0.063	3.4	0.061	2.5	0.048	1.8	0.041
7.6	0.031	5.4	0.040	4.0	0.039	2.9	0.029
12.5	0.017	9.0	0.017	6.6	0.015	4.8	0.018
20.0	0.000	14.5	0.000	10.5	0.000	7.8	0.000
		pH 7.0 and a brine concentration of 1.6%					
12.5	0.094	9.0	0.103	6.6	0.108	4.8	0.104
20.0	0.073	14.5	0.067	10.5	0.087	7.8	0.087
33.0	0.030	24.0	0.015	18.0	0.036	13.0	0.041
55.0	0.000	40.0	0.000	29.0	0.000	21.0	0.000
		pH 7.0 and a brine concentration of 2.4%					
7.6	0.063	5.4	0.057	4.0	0.063	2.9	0.061
12.5	0.045	9.0	0.044	6.6	0.047	4.8	0.044
20.0	0.026	14.5	0.027	10.5	0.017	7.8	0.026
33.0	0.000	24.0	0.000	18.0	0.000	13.0	0.000

illustrated in Figure 1 show that the F values derived from the data on can expansion are less, and the z values greater, than the F and z values for spore destruction. These results agree generally with those of Reynolds (12), who observed z values ranging from 14.5 to 27.5° F. for PA 3679 when the curves were based on the results of incubation tests of the food substrate. The results also agree with Townsend *et al.* (16), to the extent that F values are lower and z values are different when the spores are incubated in a food substrate slightly inhibitory to growth, as compared to incubation of the spores in an optimum growth medium.

The theoretical number of viable spores remaining (B) in the cans of processed cheese spread showing expansion following heat treatment at the longest heating time, and no expansion at the shortest heating time, were calculated, using the thermal destruction data in Tables 1 and 2; these are tabulated in Table 3. Values were calculated using the equation

$$\log B = \log A - \frac{U}{D}$$

The value of A was 3.3×10^6, the initial number of spores per TDT can. Values of U are those given in Table 2 for the longest heating time at which expansion of cans occurred and the shortest heating time at which there was no expansion. D values were obtained from the data in Table 1. At pH 7.0, the two values were averaged to give a D_{235} of 8.25 min. At pH 6.0, it was necessary to interpolate between pH 5.5 and 6.25 to obtain the D value of 6.25 min. D values at 220, 225, and 230° F. were calculated using the D_{235} values and a z value of 18.2° F., which is the average of the z values of all the tests recorded in Table 2. The following equation was used

$$Dt = D_{235} \cdot 10^{\frac{235 - t}{z}}$$

Data in Table 3 reveal that a relatively large number of spores are necessary to initiate gas production in the inhibitory cheese substrate. It is of interest that apparently fewer spores were required to produce can expansion in processed cheese spread at pH 7.0 and a brine concentration of 1.6% than at pH 6 and a brine concentration of 2.4%; also, that the calculated number of spores necessary to initiate can expansion decreases with increases in the processing temperature. This would seem to indicate that some inhibitory factor is partially destroyed at the higher temperature or that some limiting nutrient or other stimulating factor becomes more available.

It should be pointed out that failure of an organism to produce gas does not necessarily mean that germination and multiplication were completely

TABLE 3

Calculated number of spores in cans showing expansion at longest heating time and no expansion at shortest heating time, as influenced by pH, brine concentration, and processing temperature

pH	Temperature (° F.)	No. of spores in thousands at brine concentration of: 1.6%		2.4%	
		No growth	Growth	No growth	Growth
6.0	220	533	1,094	1,094	1,656
	225	274	733	733	1,294
	230	98	425	425	910
	235	27	186	186	562
7.0	220	331	832	832	1,429
	225	141	497	497	1,052
	230	44	227	227	692
	235	9	88	88	373

inhibited. Greenberg, Silliker, and Fatta (6) point out that high brine levels make it possible for *Cl. botulinum* to produce toxin in canned meats with no organoleptic change or gas production. Because of the possible hazards, it is suggested that processes for cheese spreads be used that will insure the destruction rather than the inhibition of potential spoilage organisms.

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