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Thermal Resistance of Putrefactive Anaerobe No. 3679 Spores in Vegetables In the Temperature Range of 250-290° F.[a, b]

W. B. Esselen and I. J. Pflug[c]
Department of Food Technology and Agricultural Engineering, University of Massachusetts, Amherst, Massachusetts

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AN INVESTIGATION OF THE THERMAL RESISTANCE of Putrefactive Anaerobe No. 3679 (P.A. 3679)[d] by Pflug and Esselen *(2, 3)* has been extended to observe thermal destruction characteristics of this organism in the temperature range of 250° to 290° F. in vegetables. Data were obtained with these spores in fresh or frozen asparagus, green beans, corn, peas, spinach, and squash. Additional data were obtained on samples of these products after having been canned in order to determine the effect of a prior heat treatment and accompanying decrease in pH value on the thermal resistance of bacterial spores.

EXPERIMENTAL

Preparation of vegetable substrates. The fresh and frozen vegetables were cut up, diluted with two parts by weight of distilled water, and pureed for 5 minutes in a Waring blender. The puree was then strained through 4 layers of cheesecloth. Twenty-five ml. aliquots of these purees were placed in sterile bottles containing glass beads and stored at 35° or 0° F. Just prior to use they were inoculated with P. A. 3679 spores at a concentration of 10,000 spores per 0.01 ml. In the case of canned vegetables, portions of the same lot of fresh or frozen product were canned in water in No. 2 size cans and processed at 240° F. according to processing schedules in National Canners Association Bulletin 26-L. A record was kept of the amount of water added to each can. When the canned products were pureed sufficient water was added to give a final dilution of one part vegetable to two parts water by weight. The pH value of each substrate was determined with a Beckman Model G pH Meter.

Determination of thermal resistance. The thermal resistance of the P.A. 3679 spores in the different vegetable substrates and in neutral phosphate buffer and distilled water was determined at temperatures of 270°, 275°, 280°, 285°, 290° F. using a thermoresistometer and procedures as previously described by Pflug and Esselen *(2, 3)*. Amounts of 0.01 ml. of the various substrates containing 10,000 spores, each were added to each thermoresistometer cup. Twenty-four samples were heated at each time and temperature interval. As least seven time intervals were used at each temperature. In some cases tests were also made at temperatures of 250° and 260° F. The heated samples were subcultured in liver broth which was pre-stratified with a mineral oil-paraffin mixture. The tubes were incubated for 8 weeks at 90° F.

Treatment of data. D values (time for 90% destruction of the spores) were calculated according to the method described by Stumbo, Murphy and Cochran *(7)*, and by the method suggested by Schmidt *(5)*. In this latter procedure, the D values are calculated on a basis of the total data acquired during an experiment. The probability of sterility is plotted against time upon arithmetic probability paper. The time corresponding to L.D. 50, or the point on the curve where the probability of a tube being viable or sterile is equal to 0.5, is assumed to represent a survival level of 0.69 spore per tube. The D value is then calculated according to the formula:

$$D = \frac{\text{Time (L.D.50)}}{\text{log initial number of spores per tube} - \log 0.69}$$

Survival and destruction end point curves were also made and extended to 250° F. The F and z values of these curves were obtained.

RESULTS AND DISCUSSION

Results obtained are summarized in Table 1 and Figures 1, 2, 3, and 4. After working with the first five fresh and frozen products (Table 1) tests were set up with new samples of vegetables to observe the effect of a prior heat treatment (canning) on the thermal resistance of P. A. 3679 spores. It is well known that during canning a decrease in pH value and other changes occur in vegetables.

Sognefest, Hays, Wheaton and Benjamin *(6)* showed that thermal processing of nonacid foods tends to lower the pH and that, in general, the higher the pH of the product before heating the greater the effect of heat in lowering the pH. The higher temperature equivalent processes caused less pH reduction than did the lower temperature processes in the range 260° to 230° F. It was further indicated that the lower the pH of a raw vegetable within the range 4.5 and 9, the lower the sterilizing value needed to insure a commercially sterile product. Thermal death time tests were carried out on fresh vegetable purees to which various amounts of HCl and NaOH were added in order to modify the natural pH values.

As has been observed by Reynolds, Kaplan, Spencer and Lichtenstein *(4)* and Schmidt *(5)*, it was found (Table 1) that z and D values calculated by the methods of Stumbo, Murphy and Cochran *(7)* and Schmidt *(5)* were generally in good agreement although some exceptions are apparent. Thermal resistance curves obtained by plotting D values [calculated by method of Stumbo, Murphy, and Cochran *(7)*] against temperatures are shown in Figures 1, 2, 3, and 4. These curves tend to follow a straight line and indicate a logarithmic order of destruction in the temperature range of 250° or 270° to 290° F.

Further examination of the data and the above curves suggested that in some products a straight line plotted in the temperature range of 250° to 270° or 275° F. had a slightly steeper slope or lower z value than such a curve plotted in the temperature range of 270° or 275° to 290° F. Because only limited amounts of data were obtained in the temperature range of 250° to 270° F., it was impossible to make a more complete analysis of these observations. However, based on such data as were available thermal resistance curves were plotted in the range of 250° to 270° F. and 270° to 290° F. and

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[b] Contribution No. 1053, of the University of Massachusetts College of Agricultural Experiment Station, Amherst, Mass.
[c] Present address: Department of Agricultural Engineering, Michigan State University, East Lansing, Michigan.
[d] A putrefactive anaerobe isolated by the National Canners Association Research Laboratories.

TABLE 1

Thermal death time characteristics of P. A. 3679 spores in pureed vegetables, distilled water, and neutral phosphate buffer in the temperature range of 250° to 290° F.

No.	Product	pH	D value (Stumbo's Method) Temperature (° F.)								D value (Schmidt's Method) Temperature (° F.)								E. P. Method	
			250	260	270	275	280	285	290	z	250	260	270	275	280	285	290	z	F	z
1	Green beans, fresh	5.85	......	......	.0714	.0308	.0260	.0108	.0078	18.4	......	......	.0536	.0310	.0216	.0115	.0072	23.3	3.20	23.0
2	Corn, frozen	7.20	......	......	.0500	.0321	.0168	.0109	.0070	21.8	......	......	.0524	.0274	.0151	.0087	.0061	20.7	2.60	22.0
3	Peas, frozen	6.95	......	......	.0824	.0388	.0190	.0121	.0078	18.8	......	......	.0842	.0372	.0195	.0166	.0079	19.0	4.20	22.0
4	Spinch, frozen	6.69	......	......	.0908	.0493	.0246	.0161	.0103	20.0	......	......	.0943	.0457	.0245	.0165	.0099	20.6	7.00	18.8
5	Hubbard squash, fresh	5.73	......	......	.0365	.0253	.0161	.0099	.0064	26.5	......	......	.0365	.0252	.0156	.0091	.0055	23.0	1.40	27.7
6	Green beans, frozen	6.03	......	......	.0529	.0320	......	.0106	.0079	22.8	......	......	.0572	.0322	......	.0113	.0072	22.1	3.00	22.2
7	Green beans, canned	5.82	......	......	.0490	.0281	......	.0095	.0061	22.0	......	......	.0517	.0288	......	.0096	.0060	21.6	2.85	19.9
8	Asparagus, frozen	6.15	......	......	.0456	.0252	.0146	.0092	.0060	22.4	......	......	.0445	.0248	.0148	.0073	.0047	20.0	2.10	24.6
9	Asparagus, canned	5.65	......	......	.0445	.0298	.0144	.0099	.0056	21.9	......	......	.0445	.0312	.0143	.0099	.0049	20.9	2.05	24.1
10	Green beans, fresh	6.00	.932	.282	.0419	.0283	.0153	.0080	.0045	16.2	.997	.262	.0404	.0277	.0163	.0084	.0044	17.1	6.0	19.6
11	Green beans, canned	5.70	.751	.196	.0416	.0229	.0129	.0090	.0055	22.2	.769	.205	.0428	.0248	.0128	.0091	.0052	17.8	4.3	19.6
12	Carrots, fresh	6.18	1.115	.234	.0530	.0373	.0190	.0114	.0064	18.4	1.000	.252	.0560	.0340	.0190	.0110	.0068	18.2	5.3	19.6
13	Carrots, canned	5.48	.777	.190	.0503	.0257	.0169	.0096	.0059	19.6	.794	.208	.0524	.0281	.0181	.0101	.0059	18.8	4.7	19.9
14	Corn, frozen	6.64	1.099	......	.0582	.0310	.0190	.0120	.0065	17.8	1.033	......	.0590	.0312	.0192	.0115	.0063	19.0	6.50	18.9
15	Corn, canned	6.48	......	......	.0506	.0270	.0118	.0105	.0063	20.5	......	......	.0472	.0273	.0157	.0108	.0068	21.3	2.30	23.7
16	Peas, frozen	6.75	......	......	.0702	.0407	.0211	.0135	.0076	21.0	......	......	.0740	.0411	.0215	.0130	.0073	19.9	4.50	20.7
17	Peas, canned	6.27	......	......	.0452	.0259	.0150	.0105	.0062	23.0	......	......	.0425	.0264	.0154	.0083	.0046	20.4	3.15	22.6
18	Spinach, frozen	6.56	.997	.254	.0629	.0353	.0155	.0098	.0064	17.5	1.100	.260	.0722	.0361	.0151	.0091	.0062	18.0	5.60	20.4
19	Spinach, canned	5.55	......	.227	.0684	.0308	.0164	.0110	.0053	18.5	......	.227	.0541	.0313	.0172	.0108	.0055	19.7	4.20	21.3
20	Distilled water		.938	.324	.0511	.0326	.0173	.0115	......	18.8	.938	.235	.0505	.0235	.0173	.0106	......	19.0	4.80	19.0
21	Neutral M/15 phosphate buffer	7.00	2.033	.723	.1860	.0931	......	.0271	.0159	18.9	1.984	.817	.192	.0957	......	.0276	.0162	17.8	14.00	19.2

their z values compared as summarized in Table 2. Curves showing these relationships of fresh carrots are presented in Figure 5. Consideration has been given to the reason for this apparent change in slope of thermal resistance curves at temperatures above 270° F. Thermal resistance data in the temperature range of 220° to

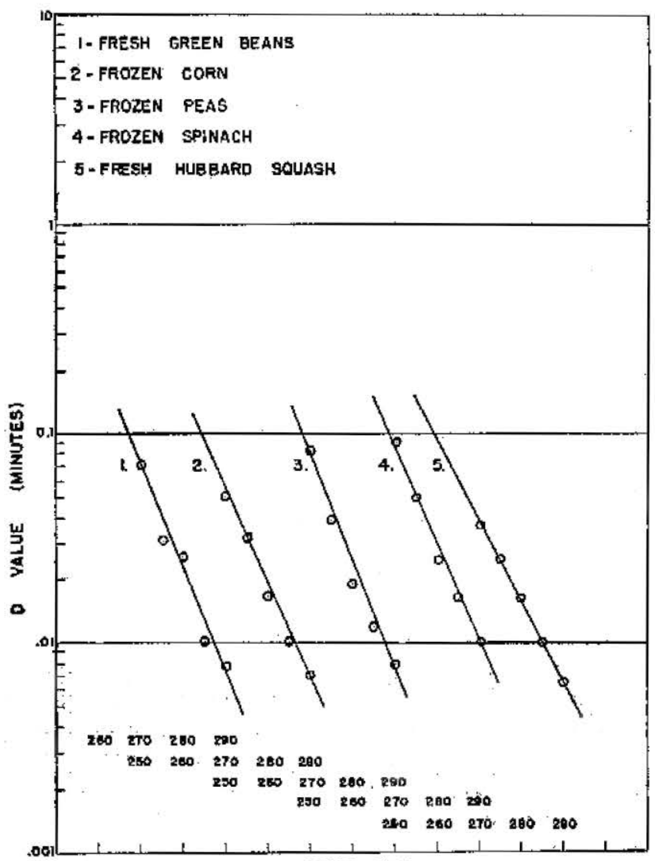

Figure 1. Thermal resistance curves for P. A. 3679 spores in fresh green beans, frozen corn, frozen peas, frozen spinach, and fresh Hubbard squash.

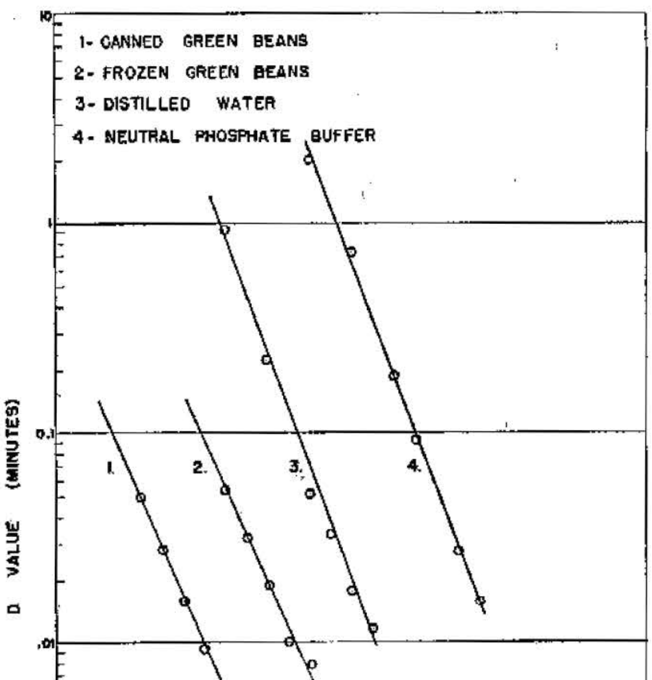

Figure 2. Thermal resistance curves for P. A. 3679 spores in canned green beans, frozen green beans, distilled water and neutral phosphate buffer.

270° F. as presented by Stumbo, Murphy and Cochran *(7)* indicated a good straight line relationship. The z values for their curves and the data as reported by others are of the magnitude of those shown in Table 2 for temperatures from 250° to 270° F. Explanations for this apparent change in z value at temperatures above

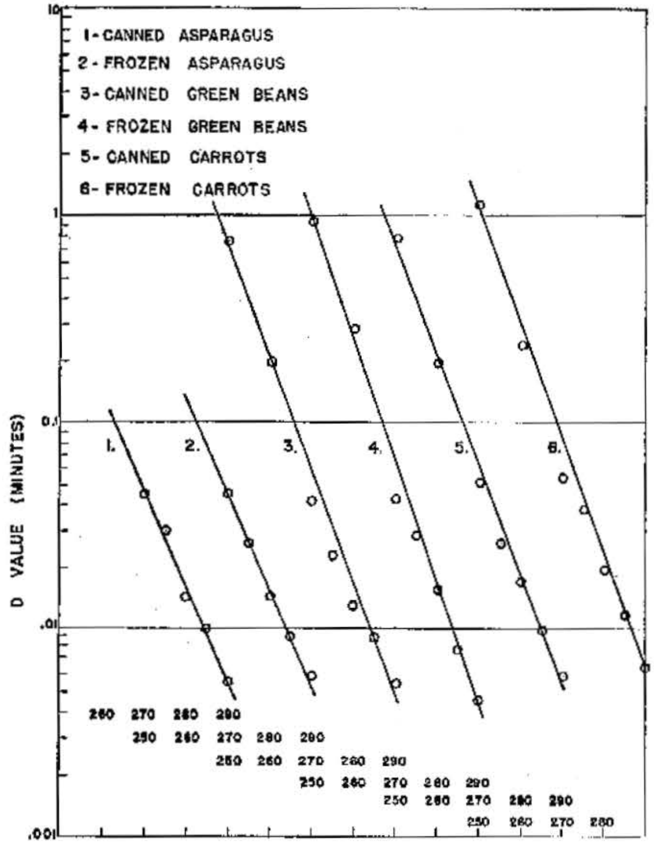

Figure 3. Thermal resistance curves for P. A. 3679 spores in canned and frozen asparagus, green beans, and carrots.

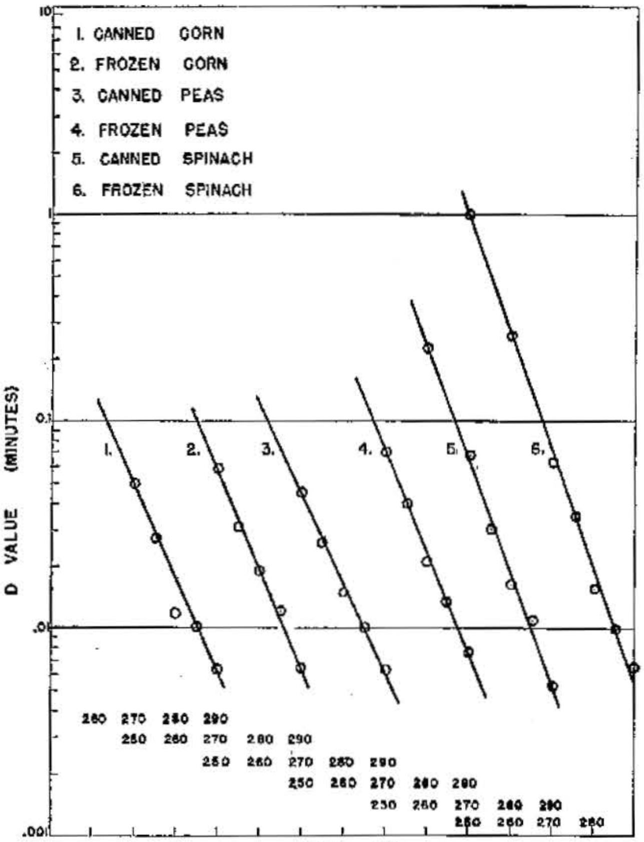

Figure 4. Thermal resistance curves for P. A. 3679 spores in canned and frozen corn, peas, and spinach.

270° F. can be postulated. It is possible that chemical changes that occur in vegetables during longer holding periods at lower temperatures may exert an influence on spores which does not occur at higher temperatures with very short holding times. Secondly, although every effort has been made to maintain heating with saturated steam in the thermoresistometer, if at the higher temperatures a dryer steam were encountered it might contribute to an increased z value as has been reported by Collier and Townsend *(1)* in the case of superheated steam.

TABLE 2

Comparison of z values of thermal death time curves of P. A. 3679 spores in the temperature ranges of 250° to 270° F. and 270° to 290° F.

Product	z value D values (Stumbo)		z value D values (Schmidt)	
	250°-270° F.	270°-290° F.	250°-270° F.	270°-290° F.
Fresh green beans	14.6	18.8	14.4	20.8
Canned green beans	16.2	22.3	15.9	22.2
Fresh carrots	15.1	21.2	15.8	21.8
Canned carrots	16.8	21.8	16.8	21.3
Frozen corn	15.7	21.4	16.2	20.8
Frozen spinach	16.5	22.7	16.7	25.8
Canned spinach	18.6	18.6	15.4	21.2
Average	16.2	21.0	15.9	22.0

Such apparent changes in z values at high temperatures do not necessarily mean that there is a deviation from a logarithmic destruction rate per se. Application of the logarithmic destruction rate theory assumes spores of uniform heat resistance heated in a medium which remains constant in composition and reaction

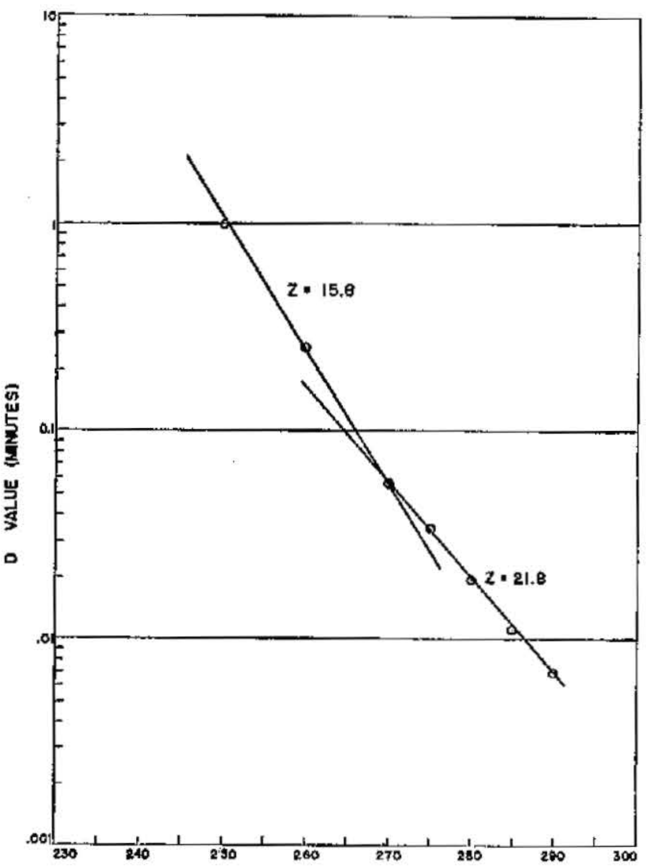

Figure 5. Thermal resistance curves for P. A. 3679 spores in fresh carrots illustrating difference in z values in temperature range of 250° to 270° F. and 270° to 290° F.

during the heat treatment. From a consideration of known and unknown changes that occur in foods during heating and the reaction rates of such changes, it is apparent that we are not dealing with a static heating substrate. Under these conditions, it seems reasonable to expect that such alterations in a food during heating could exert an influence on the apparent thermal destruction rate of bacterial spores. A comparison of the F values for fresh or frozen and previously canned vegetables illustrates the effect of extreme degrees of heating on the thermal resistance of spores in a food.

SUMMARY

Thermal resistance data for P.A. 3679 spores in fresh, frozen, and previously canned vegetables were obtained in the temperature range of 250° to 290° F. In general, the spores exhibited a lower degree of heat resistance in previously canned vegetables than in the fresh or frozen product. Spore destruction times tended to follow a semi-logarithmic destruction rate in the temperature range investigated. However, it appeared that there may be some changes in the destruction rate at temperatures above 270° F. It is suggested that such changes in spore destruction rates may be due to the influence of chemical changes occurring in food during heating.

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