J. Fd Hyg. Safety, 13(2), 143 - 148(1998)

Reestablishment of Approval Toxin Amount in Paralytic Shellfish Poison-Infested Shellfish

3. Thermal Resistance of Paralytic Shellfish Poison

Il Shik Shin† and Young Man Kim*

Faculty of Marine Biotechnology and Science, Kangnung National University, Kangnung, 210-702, Korea

**Department of Food Sciences and Nutrition, Dongeui University Pusan, 614-714, Korea*

마비성 패류독 허용기준치 재설정을 위한 연구

3. 마비성 패류독의 내열성

신일식† · 김영만*

*강릉대학교 해양생명공학부, *동의대학교 식품영양학과*

ABSTRACT — The purpose of this study was to determine the kinetics of paralytic shellfish poison (PSP) destruction at various temperature. The toxic digestive gland homogenate of blue mussel (*Mytilus edulis*), PSP crude toxin, gonyautoxin group and saxitoxin group were heated at temperature ranging from 90 to 120°C, and then the toxicities were measured in samples heated for various time intervals. The rate constant (k) of the toxic digestive gland homogenate, PSP crude toxin, gonyautoxin group and saxitoxin group were 3.28×10^{-2}, 1.20×10^{-2}, 5.88×10^{-2} and 2.58×10^{-2} at 120°C, respectively. The decimal reduction time (D-value) of the toxic digestive gland homogenate, PSP crude toxin, gonyautoxin group and saxitoxin group were 70, 192, 39 and 89 at 120°C, respectively. These results indicate that PSP crude toxin is most heat-stable of 4 types of PSP toxins and PSP toxin are more heat-stable than food poisoning bacteria and spores. The retorting condition to reduce PSP toxicity below quarantine limit (80 μg/100 g in Korea and America, 4 MU/g in Japan) could be calculated by rate constant. For example, the digestive gland homogenate having a initial toxicity of 200 μg/100 g could have toxicity below quarantine limit when heated at 90°C for 129 min., 100°C for 82 min., 110°C for 48 min. and 120°C for 28 min. These results suggest that commercial retorting condition (115°C for 70 min) in Korea is enough to reduce toxicity below quarantine limit from initial toxicity of 200 μg/100 g. From these results, the quarantine limit of PSP-infested shellfish for canning can be level up to raw score of 200 μg/100 g.

Key words □ PSP, toxic digestive gland homogenate, PSP crude toxin, GTX, STX, Rate constant, D-value

Bivalves are often toxified with paralytic shellfish poison (PSP) that is produced by some species of dinoflagellates and blocks sodium channel of nerve system.[1] PSP is accumulated in the digestive gland of bivalves, such as blue mussel and sea scallop. Contamination of commercially important shellfish with PSP poses serious problem to shellfish and related industries in Korea and other countries.

Attempts have been made to detoxify the accumulated PSP in bivalves. Of the several methods proposed for removal of PSP from contaminated shellfish, heat treatment has been most popular, although a large percentage of the incidents of PSP illness have been related to the ingestion of cooked shellfish. Prakash *et al.* reported that the total toxicity in a scallop was decreased by about 90% during canning process.[2] Noguchi *et al.* also demonstrated that a significant reduction of toxicity in the Japanese scallop occurred during canning process.[3] In the previous paper, we reported that the total toxicity in a blue mussel and oyster were decreased by about 80%

†Author to whom correspondence should be addressed.

during canning process.[4] These findings indicate that canning is useful and practical means to make PSP-infested shellfish acceptable as food. The kinetics of thermal destruction on various types of PSP toxins, however, have not been reported quantitatively.

The purpose of this study was to determine and compare the kinetics of thermal destruction on 4 types of PSP toxins at various temperature.

MATERIALS AND METHODS

Materials

Specimens of the PSP-infested blue mussel (*Mytilus edulis*) were collected from Kŏje bay, Kyoungnam Prefecture, Korea and kept below -20°C. Toxic digestive gland homogenate (41,600 μg/100 g) was prepared from PSP-infested blue mussel. The blue mussels were washed, shucked and then the digestive glands were isolated. The digestive glands were homogenized in a waring blender. The homogenate was thoroughly mixed so that all sub-samples taken from the homogenate would contain identical toxicity. Crude PSP toxin was prepared from the toxic digestive glands by A. O. A. C method.[5] Gonyautoxin (GTX) group (GTX 1-6, 2,100 μg/100 g) and saxitoxin (STX) group (STX, decarbamoyl STX and neoSTX, 1,040 μg/100 g) were prepared from the toxic digestive glands according to method of Onoue *et al.*[6] and Chang *et al.*[7]

Purification of GTX group and STX group

Toxins were extracted at room temperature with 80% ethyl alcohol acidified to pH 2.0 with hydrochloric acid. The extracts were concentrated under reduced press and washed several times with dichloromethane. The aqueous layer was adjusted to pH 4.0 with diluted sodium hydroxide solution and then treated with small quantity of activated charcoal until no toxicity was detected in the filtrate. The charcoal column was washed first with water and then with mixture of 1% acetic acid in 20% ethyl alcohol. The latter fraction was concentrated and adjusted to pH 5.5 with diluted sodium hydroxide solution. The fraction was applied to column (6.5×65 cm) of Bio-Gel P-2 (Bio-Rad Lab.). The column was eluted at a flow rate of 3.0 m*l*/min with about 1,000 m*l* of water and then with 2,000 m*l* of 0.15 M acetic acid. The pooled toxic fractions were concentrated and lyophilized, and the residue dissolved in 4 m*l* of water was applied to column (0.8×95 cm) of Bio-Rex 70 (Bio-Rad Lab., H^+ form). GTX group was eluted first with 0.03 N acetic acid, and then STX group was eluted by linear gradient elution with acetic acid (0.03~1.0 N).

Heating treatment

Thermal reduction time (TRT) experiments were carried out on the 4 types of PSP toxins by dispensing the puree into a series of 16×150 mm screw-capped culture tubes and heated at 90, 100, 110 and 120°C. In each heating experiment, two additional tubes were prepared with 14 cm CNL-type nonconducting thermocouples (O. F. Ecklund, Cape Coral, Florida) inserted through the culture tube caps and sealed with epoxy. Thus temperature in the thermal center of the tubes were monitored for thermal lag during the heating experiments. The heating experiments were done in a thermostatically-controlled ethylene glycol bath which was continuously stirred. The TRT tubes were immersed in the ethylene glycol bath at a given temperature and duplicate tubes were subsequently removed at various time intervals. The heated toxic digestive gland homogenate tubes were immediately cooled in ice water and toxin extracts prepared by the A. O. A. C. method.[5]

Toxicity assay

Toxicity was determined by a mouse bioassay method for PSP, using ICR strain male mouse weighing 19~21 g. Five mice were used for each sample tested. Toxicity and toxin composition were also assayed by the HPLC method of Oshima.[8]

PSP standard toxin

The PSP standard toxin were obtained from Ph. D. Yasukatsu Oshima (Tohoku University, Sendai, Japan).

RESULTS AND DISCUSSION

Thermal Reduction Time (TRT)

The rates of thermal reduction for toxicity of 4 types of PSP toxins at 90, 100, 110 and 120°C are shown Fig. 1. TRT curve show linearity of the semilog plot of PSP toxicity remaining versus heating time with high correlation coefficient. The PSP toxicity decreased with prolongation of heating time and elevation of temperature, and decreased first order, as observed for microorganisms. From these results, we found that the crude PSP toxin was most heat-stable of 4 types of PSP toxins.

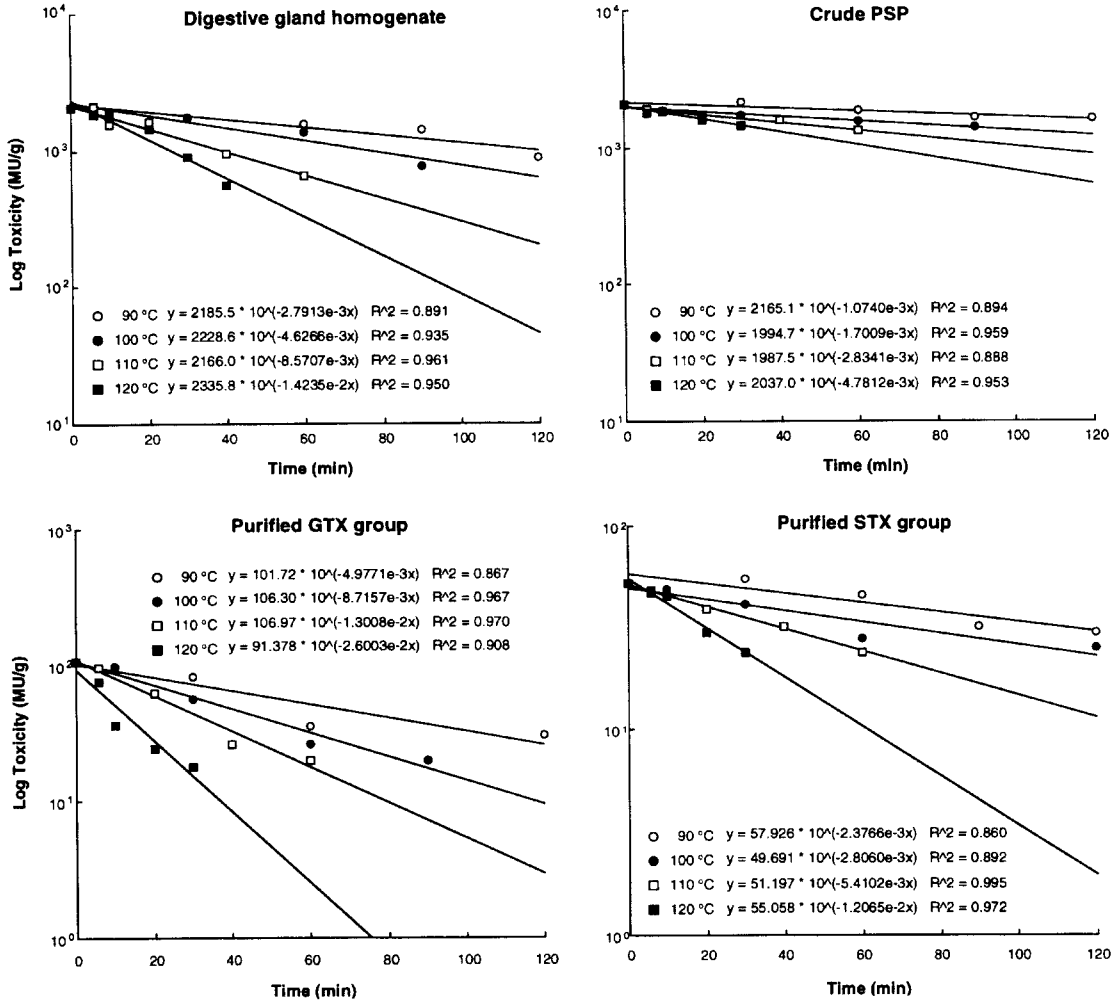

Fig. 1. thermal reduction time (TRT) curve of PSP.

Rate constant (k)

To determine the decimal reduction time (D-value) and retorting condition for PSP in canning process, the rate constant was calculated by using the data of TRT curve. The rate constant (k) for 4 kinds of PSP toxins are listed Table 1, and calculated from the linear regression equations for each curve where :

$$\text{Log}\frac{N}{N_0} = -\frac{k}{2.303} \times t$$

k: Rate constant (sec^{-1})
N_0: Initial toxicity
N: Toxicity after heating for t
t: Heating time

Table 1. Rate constant (k) of various types of PSP toxins from toxic blue mussel

Temperature (1/T, $°K \times 10^{-3}$)	Rate constant (k, se^{c-1})			
	Digestive gland homogenate	Crude PSP toxin	GTX group	STX group
2.75	7.12×10^{-3}	2.08×10^{-3}	1.04×10^{-2}	4.58×10^{-3}
2.68	1.12×10^{-2}	4.16×10^{-3}	1.84×10^{-2}	6.10×10^{-3}
2.61	1.92×10^{-2}	7.33×10^{-3}	2.76×10^{-2}	1.29×10^{-2}
2.54	3.28×10^{-2}	1.20×10^{-2}	5.88×10^{-2}	2.58×10^{-2}

The lower rate constant, the higher heat-stable. The rate constant of toxic digestive gland homogenate, PSP crude toxin, GTX group and STX group were 3.28×10^{-2}, 1.20×10^{-2}, 5.88×10^{-2} and 2.58×10^{-2} at 120°C, respectively. These results indicate that PSP crude toxin is most heat-stable and GTX group is most heat-labile. The rate constant of 4 types of PSP toxins also showed linear regression with high correlation coefficient (Fig. 2).

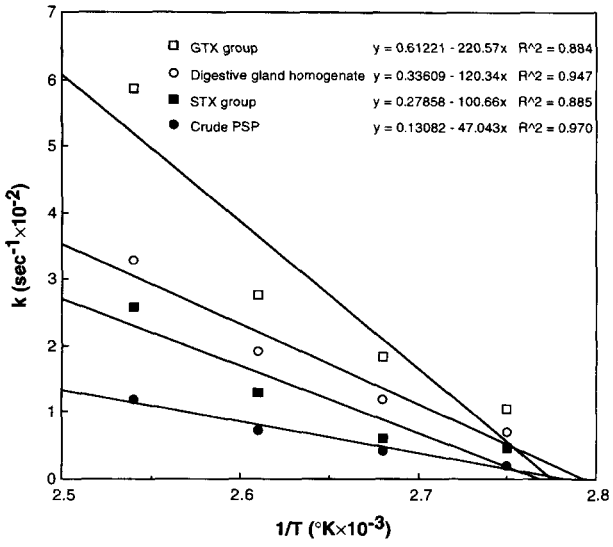

Fig. 2. Rate constant (k) for various type of PSP from blue mussel.

The retorting condition can be determined from thermal resistance of GTX group, because the toxic bivalves of Korea, such as blue mussel and oyster, contain mainly GTX group. However, STX group which are higher toxic PSP is formed from GTX group, such as GTX 5 and GTX 6 which are lower toxic PSP, when PSP toxins were heated above 100°C and at neutral pH. The retorting condition, therefore, should be determined from the thermal resistance of shellfish meat homogenate.

The retorting condition to reduce toxicity below quarantine limit (80 μg/100 g in Korea and America, 4 MU/g in Japan) of PSP could be calculated by rate constant. For example, the digestive gland homogenate having a initial toxicity of 200 μg/100 g could have toxicity below quarantine limit when heated at 90°C for 129 min., 100°C for 82 min., 110°C for 48 min. and 120°C for 28 min. (Table 2). These results suggest that commercial retorting condition (115°C for 70 min) in Korea is enough to reduce toxicity below quarantine limit from initial toxicity of 200 μg/100 g.

In the current control program of Food Sanitation Law of Korea, when PSP exceed 80 μg/100 g of edible meat in any area, all areas in its class are closed to harvest shellfishes for all purpose. This might provide a wide margin of safety to consumer but this would reduce many fishermen and processor's income. Possibly the regulation should be relaxed somewhat, because there are evidences from this study that raw shellfish with toxicity of 200 μg/100 g produces can scored less than quarantine limit. In Canada, raw shellfish with toxicity of 80 to 160 μg/100 g has been permitted for only canning since 1971. If the final toxicity of cans were below 80 μg/100 g, the cans can be released for marketing.[2)]

From these results, the quarantine limit of PSP-infested shellfish for canning can be level up to raw score of 200 μg/100 g.

Table 2. Retorting condition to reduce below quarantine limit from initial toxicity of 200 μg/100 g

PSP toxin	Heating time (min.)			
	90°C	100°C	110°C	120°C
Digestive gland homogenate	129	82	48	28
Crude PSP toxin	440	220	125	76
GTX group	88	50	33	16
STX group	200	150	71	36

Quarantine limit: 80 μg/100 g in Korea and America, 4 MU/g in Japan

Decimal reduction time (D-value)

The D-values for 4 types of PSP toxins calculated by rate constant are listed in Table 3. The D-values of crude PSP toxin were higher at each temperature than any other toxins. These results also indicate that crude PSP toxin is most heat-stable.

The D_{120}-values of PSP toxins were compared with D_{121}-values of bacteria and food component (Table 4). It would appear that PSP toxins are much more heat-stable than food poisoning bacteria and spores.

z-value

A thermal destruction plot of D-values versus heating temperature were shown Fig. 3. The z-values for PSP toxins were determined as -1/slope of the thermal destruction time (TDT) curve and were found to be little different among 4 types of PSP toxins.

The z-values of PSP toxins were compared with those of bacteria (Table 5). We found that PSP toxins were

Table 3. Decimal reduction time of various types of PSP toxins from blue mussel

Temperature (°C)	Decimal reduction time (D-value, min.)			
	Digestive gland homogenate	Crude PSP toxin	GTX group	STX group
90	323	1,107	221	503
100	192	554	125	378
110	120	314	83	179
120	70	192	39	89

Table 4. D_{121}-values for PSP toxins and various food poisoning bacteria

Food	Component	pH	D_{121} (min.)	Reference
Blue mussel	PSP toxin[a)]			
	Digestive gland homogenate	6.8	7.	Present study
	Crude PSP toxin	3.0	192	〃
	GTX group	5.5	39	〃
	STX group	5.5	89	〃
N.S.[b)]	*Clostridium botulinum* A, B spores	7.0	0.1~0.2	9
N.S.	*Bacillus stearothermophilus* spores	>4.5	4.0~5.0	9
N.S.	Putrefactive anaerobe 3679	>4.5	2.47	9
Milk	*Staphylococcus* enterotoxin B	6.5	9.4	10

[a)]D-values of PSP toxins are at 120°C
[b)]Type of food not stated in reference

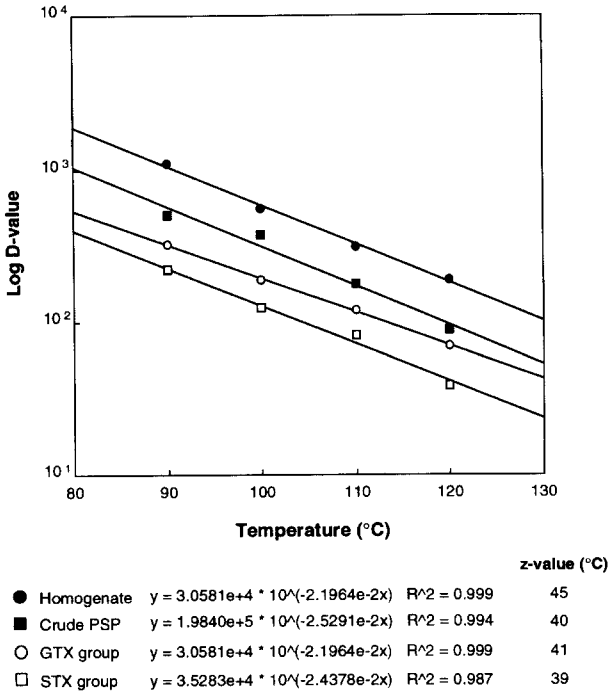

Fig. 3. TDT curve for various type of PSP from blue mussel.

also much more heat-stable than those of food poisoning bacteria and spores.

CONCLUSION

1. The crude PSP toxin extracted by 0.1 N HCl was most heat-stable among 4 types of PSP toxins from rate constant, D-value and z-value.

2. The PSP toxins were more heat-stable than food poisoning bacteria and spores from D-value and z-value.

3. By the rate constant, digestive gland homogenate having an initial toxicity of 200 μg/100 g had a toxicity below quarantine limit (80 μg/100 g) when heated at 90°C for 129 min., 100°C for 82 min., 110°C for 48 min. and 120°C for 28 min..

4. By the retorting condition deduced from rate constant, commercial retorting condition (115°C for 70 min) in Korea is enough to reduce toxicity below quarantine limit from initial toxicity of 200 μg/100 g.

5. From these results, the quarantine limit of PSP-infested shellfish for canning can be level up to raw score of 200 μg/100 g.

Table 5. z-values for PSP toxins and various food poisoning bacteria

Component	z-value (°C)	Reference
PSP toxin[a)]		
Digestive gland homogenate	45	Present study
Crude PSP toxin	40	〃
GTX group	41	〃
STX group	39	〃
Clostridium sporogenes	8~11	9
Clostridium botulinum	9~11	9
Clostridium thermosaccharolyticum	9.5~10	9
Bacillus stearothermophilus	7~10.5	10
Bacillus subtilis	6.5	
Bacillus coagulans	10	

ACKNOWLEDGMENTS

The authors wish to express their gratitude to Dr Y. Oshima and Mr. K. Yasumoto, Professor of Faculty of Agriculture, Tohoku University in Japan, for helping the use of HPLC system and standard toxins. This study was supported by grants from R&D Promotion Center for Agriculture, Forestry and Fishery of Korea Rural Economic Institute.

국문요약

마비성패류독(Paralytic shellfish poison, PSP)에 의하여 독화된 패류의 유효이용에 대한 자료를 제공하고자 독화된 진주담치의 중장선 균질육, 0.1N HCl로 추출한 조독소 용액, 중장선으로부터 정제한 gonyautoxin(GTX) group과 saxitoxin(STX) group 등 4가지 형태의 PSP 독소에 대한 내열성을 조사하였다. 독화된 진주담치의 중장선육 균질액, 산추출 조독소액, GTX group, STX group 등 4 종류의 반응속도상수는 120°C에서 3.28×10^{-2}, 1.20×10^{-2}, 5.88×10^{-2}, 2.58×10^{-2}이었으며 4 종류의 독소 중 0.1 N HCl로 추출한 조독소용액의 D-value가 가장 높았다. 반응속도상수를 이용한 살균온도 산정에 있어서, 최초 독력이 200 μg/100 g인 독화된 진주담치육의 경우, 독력을 마비성패류독 규제치인 80 μg/100 g으로 감소시키는데에는 90°C에서는 약 129분, 100°C에서는 약 82분, 110°C에서는 약 48분, 120°C에서는 약 28분이 걸렸다. 이러한 결과는 최초 독력이 200 μg/100 g인 패류의 경우, 통조림 살균공정 후 잔존 독력이 규제치인 80 μg/100 g 이하로 감소시키는데에는 현재의 살균조건(115°C, 70분)으로는 충분하다는 것을 입증한다.

REFERENCE

1. Schantz, E.J.: Chemistry and biology of saxitoxin and related toxins. *Ann. New York Acad. Sci.* **479**, 15 (1985).
2. Prakash, A., Medcof, J.C. and Tennant, A.D.: Paralytic shellfish poisoning in eastern Canada. *Fish. Res. Bd. Canada.* **177**, 1-87 (1971).
3. Noguchi, T., Ueda, Y., Onoue, Y., Kono, M., Koyama, K., Hashimoto, K., Seno, Y. and Mishima, S.: Reduction in Toxicity of PSP Infested Scallops during Canning Process. *Bull. Japan. Soc. Sci. Fish.* **46**(10), 1273-1277 (1980) (in Japanese).
4. Kim, Y.M., Choi, S.H., Kim, S.J., Suh, S.B., Pyun, H.S., Chang, D.S. and Shin, I.S.: Studies for Reestablishment of Approval Toxin Amount in Paralytic Shellfish Poison-Infested Shellfish. 1. Toxicity Change in Paralytic Shellfish Poison-Infested Blue mussel, *Mytilus edulis* and Oyster, *Crassostrea gigas* during Boiling and Canning Processes. *J. Korean Fish. Soc.* **29**(6). 893-899. (1996).
5. A.O.A.C. Paralytic shellfish Poison. *In Official Methods of Analysis of the Association of Official Analytical Chemists*, 15th Ed (Kenneth, H ed) Association of Official Analytical Chemists Inc. Virginia, pp. 881-882 (1990).
6. Onoue, Y., Noguchi, T., Maruyama, J., Ueda, Y., Hashimoto, K. and Ikeda, T.: Comparison of PSP composition between toxic oyster and *Protogonyaulax catenella* from Senzaki bay, Yamaguchi Prefecture. *Bull. Jap. Soc. Sci. Fish.* **47**(10), 1347-1350 (1981).
7. Chang, D.S., Shin, I.S., Cho, H.R., Park, M.Y., Pyeun, J. H. and Park, Y.H.: Studies on distribution, characterization and detoxification of paralytic shellfish poison (PSP) in Korea. 2. Purification and characterization of PSP extracted from cultured sea mussel, *Mytilus edulis*. *Bull. Korean Fish. Soc.* **21**(3), 161-168 (1988).
8. Oshima, Y.: Postcolumn derivatization liquid chromatographic method for paralytic shellfish toxins. *J. AOAC Inter.* **78**(2), 528-532 (1995).
9. Marth, E.H. (ed): Standard methods for the examination of dairy products, 14th ed. Am. Public Health Assoc., Washington, D. C. (1978).
10. Esty, J.R. and Williams, C.C.: Heat resistance study 1. A new method for determination oh heat resistance of bacterial spores. *J. Infec. Dis.* **34**, 516-528 (1924).