Thermal Degradation of Partially Purified Paralytic Shellfish Poison Toxins at Different Times, Temperatures, and pH

W.M. INDRASENA AND T.A. GILL

ABSTRACT: Mixtures of purified and partially purified paralytic shellfish poisoning (PSP) toxins including C1/2 and B1 toxins, gonyautoxins 1-4 (GTX), neosaxitoxin (NEO), and saxitoxin (STX) were heated at different temperatures (90 to 130 °C), heating times (10 to 120 min), and pH (3 to 7) and analyzed by HPLC. C toxins declined rapidly at low pH, and GTX 1/4 toxins decreased at high temperatures and at high pH. GTX 2/3 increased initially at low pH and then declined with subsequent heating, whereas STX increased consistently at pH 3 to 4. The integrated total specific toxicity declined at high pH (6 to 7). The kinetics of thermal destruction were 1st order, and the efficacy of thermal destruction was highly dependent on pH, with rapid thermal destruction of carbamate compounds at higher pH. D values of carbamate toxins decreased with increasing temperature at high pH. Heating at low pH resulted in conversion of least toxic compounds to highly toxic compounds.

Key Words: PSP toxins, HPLC, kinetics

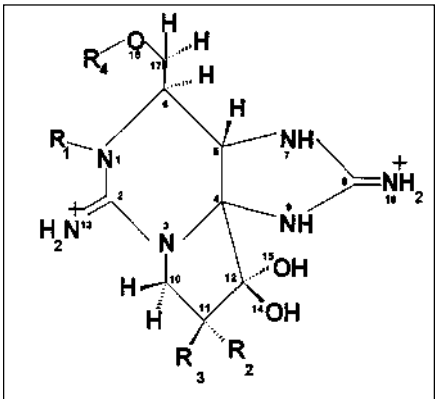
Introduction

STX AND ITS ANALOGUES (Fig. 1) PRODUCED by some dinoflagellates and cyanobacteria are among the most toxic low-molecular-weight compounds known (Laycock and others 1997). The organisms that feed on them can accumulate the toxins in high concentrations and transmit them to higher trophic levels through the food chain. These toxins block the sodium channels of the central nervous system causing paralytic shellfish poisoning (PSP). PSP poses a most serious threat to public health due to the extreme toxicities of the toxins involved (Chen and Chou 1998) with no known antidotes. Carbamate toxins such as saxitoxin, neosaxitoxin, gonyautoxins, and the N-sulfocarbamoyl toxins such as C toxins and B toxins are found in most of the toxic dinoflagellate types in varying amounts. These toxins have different toxicities, carbamate toxins being the most toxic, whereas the sulfamate toxins are the least neurotoxic to the humans.

Natural detoxification of contaminated shellfish has been a difficult task because different shellfish have different rates of toxin release. Although the toxicity can be reduced slowly by the normal process of home cooking such as boiling or frying (Medcof and others 1947, Quayle 1969), the total toxicity was reported to be reduced only by 70% after cooking for 40 min. Prakash and others (1971) indicated that steaming and frying reduced the toxicity more than boiling, whereas retorting at 121.1 °C instead of 104.4 °C reduced the final toxin levels by 25% to 50%. Lawrence and others (1994) indicated that the total toxicity of the toxic lobster hepatopancrease could be reduced by 65% during normal cooking (boiling and steaming). The STX and GTX 2/3 (combined) levels could be decreased by 60% and 90% to 100%, respectively. However, Desbiens and Cembella (1997) indicated that 1/3 of the samples became more toxic due to toxin interconversions after the lobsters were cooked in hot steam for 20 min. Mild acid hydrolysis of C2 toxins liberates inorganic sulfate and GTX 3, whereas C1 toxins that are epimers of the 11α-isomer C2 can be converted to GTX 2 by the reductive cleavage of the O-sulfate group (Shimizu 1984; Laycock and others 1995), and GTX 2/3 and NEO also can be hydrolysed to STX during heating under mild acidic conditions (Shimizu 1984) (Fig. 2).

Noguchi and others (1980a, 1980b) reported that the toxicity of PSP-infested scallops can be reduced by 90% by canning at 122 °C for 22 min. Studying the effect of heating (70 to 110 °C) and pH (6 to 8) on the toxicity of scallops, Asakawa and Takagi (1983) found that the PSP toxicity was decreased by 87% after heating at 110 °C and at pH 8 for 30 min. Asakawa and others (1986) reported conversions of GTXs into STX along with detoxification during canning.

The total toxicity in heated (100 and 121 °C) mussels, *Mytilus edulis*, over a pH range of 1 to 6 was studied by Chang and others (1988), and it was found that PSP toxins were more stable at low pH. Berenguer and others (1993) reported that the total PSP toxicity of the raw material of naturally contaminated *Acanthocadia tuberculatum* was decreased by about 95% after canning in salt water (40g/L) at 115 °C for 45 min.



	Molecular weight	R_1	R_2	R_3	R_4
STX	301.31	H	H	H	$CONH_2$
B1 (GTX5)	380.36	H	H	H	$CONHSO_3^-$
GTX 2	396.36	H	OSO_3^-	H	$CONH_2$
C1 (epi GTX6)	475.41	H	OSO_3^-	H	$CONHSO_3^-$
GTX 3	396.36	H	H	OSO_3^-	$CONH_2$
C2 (GTX8)	475.41	H	H	OSO_3^-	$CONHSO_3^-$
NEO	317.31	OH	H	H	$CONH_2$
B2 (GTX6)	396.36	OH	H	H	$CONHSO_3^-$
GTX 1	412.36	OH	OSO_3^-	H	$CONH_2$
C3	491.41	OH	OSO_3^-	H	$CONHSO_3^-$
GTX 4	412.36	OH	H	OSO_3^-	$CONH_2$
C4	491.41	OH	H	OSO_3^-	$CONHSO_3^-$
dc-STX	258.28	H	H	H	H
dc-GTX 2	353.33	H	OSO_3^-	H	H
dc-GTX 3	353.33	H	H	OSO_3^-	H
dc-NEO	274.28	OH	H	H	H
dc-GTX 1	369.33	OH	OSO_3^-	H	H
dc-GTX 4	369.33	OH	H	OSO_3^-	H

Fig. 1—Structures of PSP components (GTX = gonyautoxin, STX = saxitoxin, NEO= neosaxitoxin, dc = decarbamoyl)

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Ohta and others (1992) applied coextrusion processing to detoxify PSP toxins in scallops and found that there were 81.6% to 82.2% and 85.6% to 97.8% reductions at 130 and 170 °C, respectively. Gill and others (1985) were the first to report the kinetics of the thermal destruction of PSP toxins, indicating that they were 1st order, and a 90% reduction of total toxicity in soft-shell clams (pH = 6.8) could be achieved by heating for 43 min at 132.2 °C or 193 min at 104.4 °C (for example, the '$D_{121.1}$' and 'z' values were 71.4 min and 42 °C, respectively.) The kinetics of the thermal degradation of some individual PSP toxins were studied by Nagashima and others (1991) who reported that the kinetics were 1st order, although the toxicity increased by 50% due to toxin interconversions during heating at 100 °C for 30 to 60 min.

A more systematic and detailed study on the combined effects of pH, heating temperature and time, on the thermal degradation of individual PSP components in scallop digestive glands was done by Indrasena and Gill (1999) who reported that the kinetics of the thermal degradation of STX, NEO, and GTX 2/3 toxins were 1st order. The highest D and z values were reported for STX and lowest values for GTX 2/3. The degradation kinetics were not reported for GTX 1 and 4 nor the C toxins. In addition, the degradation rates may change according to the type of ingredients in the matrix or medium (Asakawa and Takagi 1983). Thus, the present study was carried out to gather more information on the kinetics of partially purified standard PSP toxins in buffer (pH 3 to 7), especially GTX 1/4 and C toxins, in a medium without a matrix.

Materials and Methods

Buffering and heating

Unknown amounts of purified and partially purified PSP toxins including STX, NEO, GTX 1-4, C toxins (C1 and C2), and B toxins (B1) were all mixed together and diluted with 0.01N acetic acid. The toxins were obtained from the National Research Council (Institute of Marine Biosciences, Halifax, Nova Scotia.) One-mL aliquots of the mixture were transferred into 6-cm screw-cap culture tubes, and 1 mL of citrate/phosphate (1.2M) buffer (pH of 3, 4, 5, 6, or 7) was added to each tube to adjust sample pH. Another set of tubes was prepared using 1 mL of double distilled, deionized water instead of buffer (NB) for each temperature level. The sample tubes were prepared in the same manner for all temperature levels. The tubes were thoroughly mixed and flushed with nitrogen before sealing.

The tubes were heated in a thermostatically controlled oil bath, and temperatures were monitored using thermocouples inserted through the screw caps. The oil bath was heated to the required temperature. Duplicate tubes of each pH treatment were removed after 10, 20, 40, 60, 80, 100, and 120 min and immediately transferred into an ice bath. Heated/cooled samples were removed for analysis as quickly as possible.

Analysis of toxins by high performance liquid chromatography

PSP toxins were determined with a Waters high performance liquid chromatograph equipped with 2 Model 510 pumps, an auto injection system, and a Shimadzu Model Rf 535 fluorescence detector (338 nm excitation, 400 nm emission). Samples were run in the HPLC using a Whatman PRP-1 column (15 cm x 4.1 mm) packed with 10ìm beads using a binary elution gradient (mobile phase A: water with 1mM hexane and heptane sulfonic acid, mobile phase B: acetonitrile with hexane and heptane sulfonic acid) according to Sullivan and Wekell (1987). The HPLC was equipped with a dual reagent post column reaction system set up to mix the effluent stream with nitric acid (0.75M), and periodic oxidant (5N sodium hydroxide, 5mM periodic acid, and 0.5M ammonium hydroxide) was used prior to the fluorescence detection. Individual toxins were identified by running a standard mixture of PSP toxins and were quantified using authentic PSP toxins obtained from the National Research Council. Capillary electrophoresis-mass spectrometry (CE-MS) according to the method of Locke and Thibault (1994) was used to confirm some compounds after heating. Model 270A capillary electrophoresis system was used with untreated fused-silica-capillary column. The toxin levels analyzed by the HPLC were mathematically converted to integrated total specific toxicities (Schantz 1986; Sullivan and others 1985).

Kinetics of thermal destruction

Decimal reduction times and z values for each toxin component were calculated according to Stumbo (1973). The best-fitted mathematical model for the destruction of each PSP component was determined by the MINITAB Version 12.2 for Windows in the Dalhousie University (DalTech) mainframe computer. The destruction of toxins at different time intervals was statistically compared by analysis of variance (ANOVA) via multiple regression using indicator variables.

Results and Discussion

THE STANDARD TOXIN MIXTURE CONtained C1 and 2, B1, GTX 1- 4, NEO, and STX (Fig. 3). The kinetics of the degradation of most of the toxins were found to be 1st order and comparable with the degradation patterns of individual toxins in the scallop homogenate reported in a previous study (Indrasena and Gill 1999). Gill and others (1985) and Nagashima and others (1991) also reported that the kinetics of the thermal degradation of PSP toxins were 1st order. However, the rate of degradation depends not only on the type of toxins but also on the heating temperature, time, and pH of the heating medium.

Effect of pH

With gentle heating at 90 °C, the amount of C1/C2 toxins declined gradually ($P \leq 0.01$) over an initial time period of 2 h. Unlike other individual toxins in the mixture, C toxins declined rapidly at low pH with the fastest decrease at pH 3 during heating for 2 h followed by pH 4, NB (control sample with no buffer), 5, 6, and 7 (Fig. 4a to 4c). The rate of conversion or de-

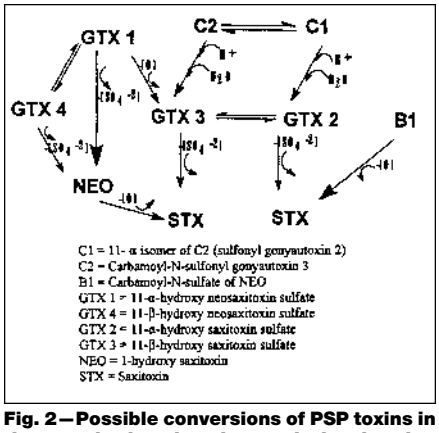

Fig. 2—Possible conversions of PSP toxins in the standard toxin mixture during heating (Shimizu 1984; Laycock and others 1995)

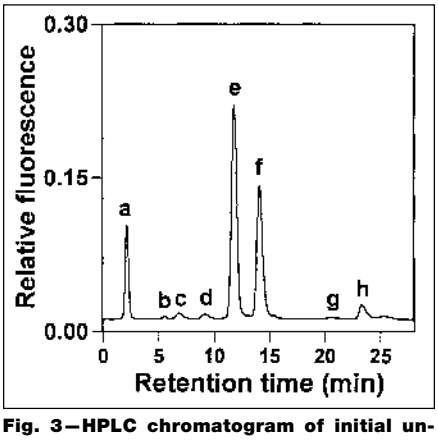

Fig. 3—HPLC chromatogram of initial unheated mixture of PSP toxins (a = C1-2, b = GTX 4, c = GTX 1, d = B1, e = GTX 3, f = GTX 2, g = NEO, h = STX)

struction was much faster at higher temperatures.

The same general pattern was observed when the temperature was increased from 90 °C through 120 °C. However, at pH 6, C toxins did not change much when heated at 110 to 120 °C for longer periods (>2 h). At pH 7, these toxins actually tended to increase after 80 min heating at 110° and 120 °C. This observation may be due to the co-elution of some thermally denatured PSP toxins in the reaction mixture and/or conversion of some other toxins in the mixture into C toxins. The increase in the C-toxin peak was more pronounced at 130 °C throughout the whole range of pH (3 to 7). Indrasena and Gill (1999) indicated that the C-toxin peak in scallop homogenates also increased gradually with increasing temperature, especially at neutral pH, possibly by the co-elution of thermally degraded compounds. C1/C2 toxins were found to be the most heat labile compounds in the standard toxin mixture, and their sensitivity to heating was significantly affected not only by the heating temperature and time but also by the pH.

The levels of GTX 1 and 4 gradually declined during heating, at 90 °C for 2 h (Fig. 5a). Unlike C toxins, GTX 1/4 degraded most at the highest pH. Thermal destruction was greatest at higher temperatures. Although it is tempting to conclude that the gradual reduction in the GTX 1/4 levels at low pH levels was due to thermal degradation, it may possibly also be due to their conversion into more toxic NEO, however no mass spectral evidence was gathered in order to confirm or deny this hypothesis. Since GTX 1 and 4 are epimeric 11-hydroxyneosaxitoxin sulfates, they can be converted to NEO by the reductive cleavage of the O-sulfate groups when heated in mild acid (Fig. 2). However, when heated from 90 °C through 130 °C, the level of GTX 1/4 declined rapidly (P_ 0.01) with the highest rates observed at pH 6 to 7 (Fig. 5b and 5c).

Unlike GTX 1/4, GTX 2 and 3 increased steadily ($P \leq 0.05$) at low pH levels (3 to 5) when heated at 90 °C for 40 min (not shown), whereas at pH 7, there was no increase. GTX 2/3 gradually increased at low pH levels when heated at 110 °C and then declined (not shown). Low temperature (90 °C and 110 °C) heating of GTX 2/3 in scallop tissues gave similar results (Indrasena and Gill 1999). This gradual increase may be due to the conversion of C1/C2 to GTX 2/3 by the removal of sulfo groups by carbamoylation. The decrease of the GTX 2/3 may be due to thermal degradation as well as possible conversion into more toxic STX. However, at higher pH, the reduction may also be due to thermal destruction rather than conversion. The amount of GTX 2/3 declined slowly after 20 min of heating at 120 °C at low pH (3 to 4 and control), whereas the sample at pH 5 did not change very much. These toxins gradually decreased at pH 6 to 7, and this decrease was more pronounced when heated at 130 °C. However, it is difficult to determine the fate of toxins during heating at moderately high temperatures (100 to 110 °C) due to possible simultaneous conversions and destruction.

The changes in B1 toxin were difficult to quantify during heating due to the small quantities present in the samples. It is possible that these toxins may be converted to STX at low pH levels (Fig. 2). An extraneous peak was observed and eluted between B1 and GTX 3. This may be one of the thermally degraded components of PSP or decarbamoyl counterparts of GTXs. The degradation patterns of NEO at different temperatures were quite similar to those of STX, and highest degradation rates were noticed at higher pH.

The amount of STX increased slowly and gradually (not shown) after 40 min heating at 90 °C at pH 3 to 4, whereas at pH 6 to 7, there was no noticeable increase or decrease. A similar pattern was noticed when toxins were heated at 90 °C through 110 °C except the STX content started to decline after 20 min at pH 6 to 7. When the temperature was further increased to

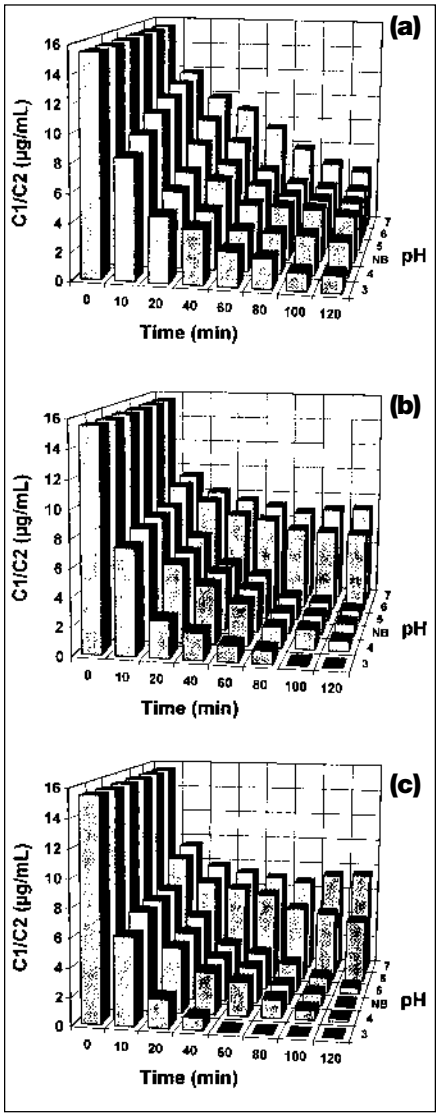

Fig. 4—(a) Thermal degradation of C1/C2 in the standard toxin mixture heated at 90 °C at different time and pH. (b) Thermal degradation of C1/C2 in the standard toxin mixture heated at 110 °C at different time and pH. (c) Thermal degradation of C1/C2 in the standard toxin mixture heated at 120 °C at different time and pH

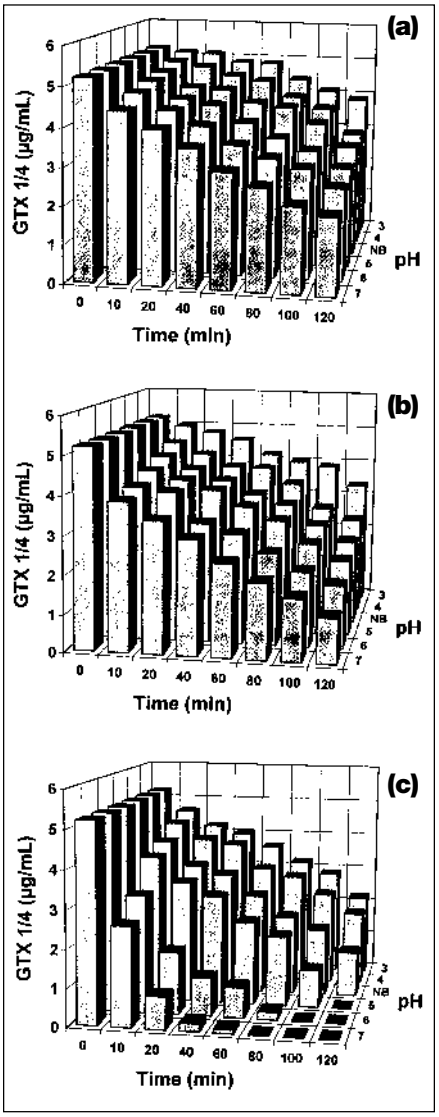

Fig. 5—(a) Thermal degradation of GTX 1/4 in the standard toxin mixture heated at 90 °C at different time and pH (b) Thermal degradation of GTX 1/4 in the standard toxin mixture heated at 110 °C at different time and pH (c) Thermal degradation of GTX 1/4 in the standard toxin mixture heated at 130 °C at different time and pH

120 °C, the amount of STX started to decline after 10 and 40 min at high pH (6 to 7) and low pH (3 to 4), respectively, whereas at 130 °C, STX content declined at all pH with fastest rates at high pH. Indrasena and Gill (1999) observed the similar degradation pattern of STX in a heated toxic scallop homogenate under similar conditions. However, STX in the standard PSP mixture degraded much more slowly than that in the scallop homogenate. In addition to this inherent resistance to heat, STX may be generated through heating by conversion of GTX 2/3, B1 toxin, and NEO during mild heating at low pH levels. This may in part explain the slower-degradation rate of STX in the standard toxin mixture. GTX 2 and 3 can be converted to STX by heating in the acid by reductive elimination of the sulfonyl group (Shimizu 1988). NEO in the medium also can be converted to STX by reductive cleavage of N-hydroxyl group.

Integrated specific toxicity

The theoretical integrated specific toxicities may be determined mathematically as mouse units (MU/mL) using the conversion factors reported by Schantz (1986) and Sullivan and others (1985). The D values for total toxin were much higher at low pH and temperatures. Figures 6a to 6c show the change in the total integrated specific toxicities at 90, 110, and 130 °C. The total toxicity increased slightly at pH 3 to 4 at 90 °C, whereas there was a gradual decline at pH 6 to 7. However, despite the possible toxin interconversions, the total toxicity gradually declined with increasing heating times and temperature with the maximum decrease at neutral pH (6 to 7). It was also observed that the total toxicity did not change much at low pH (pH 3 to 4), even at higher temperatures. Indrasena and Gill (1999) showed that the theoretical integrated specific toxicities calculated from HPLC data obtained for PSP-containing scallop homogenate heated under identical conditions were highly significantly correlated ($P \leq 0.01$) with the actual specific toxicities obtained from the mouse neuroblastoma cell bioassay. This assay (Jellet and others 1992) is based upon the sodium channel blocking properties of PSP toxins and is now commercially available (Jellett Biotek Ltd., Dartmouth, Nova Scotia, Canada). The data indicated the reliability of the Sullivan and Wekell (1987) method for the determination of toxicity using chromatographic data. Theoretical integrated specific toxicities calculated for heated toxins without a matrix under similar conditions in the present study also had the same general pattern. Chang and others (1988) also indicated that the total toxicity increased at pH 3 followed by a gradual decrease with increasing pH. Gill and others (1985) and Asakawa and Takagi (1983) reported the gradual decrease in total toxicity during heating around neutral pH.

Reaction kinetics

Decimal reduction time (D value), which is defined as the time (min) required to destroy 90% of a substance or organism at a given temperature, was calculated for each toxin type at each pH and temperature level as the inverse negative slope of the survivor curves. This concept has been widely used in the thermal destruction of a variety of compounds important in the food industry, and the D value can vary widely with the physico-chemical nature of the compounds in the medium, temperature, and various other factors (Banwart 1989). The higher the D value, the more thermally stable the compounds are. Gill and others (1985), using mouse bioassay data, indicated that the kinetics of thermal destruction of PSP in heated soft-shell clam homogenates (pH = 6.8) was 1st order, and the D values decreased semi-logarithmically with increasing temperature. Indrasena and Gill (1999) previously reported D values for some individual toxins in scallop homogenate heated at different temperatures and pH 3 to 7.

TDT curves for C1/2 and GTX 1/4 toxins are shown in Fig. 7 and 8, respectively, and the D values calculated for C1/2 and GTX 1/4 toxins from the linear regression equations of the survivor curves are shown in Tables 1 and 2. The D values of C toxins were smaller at low pH than those at higher pH (Table 1). Thus, 90% reduction of C1/C2 could be obtained by heating at 120 °C for 22.3 min at pH 3 or for 67.1 min at pH 7. The decimal reduction times clearly indicate that GTX 1 and 4 were more heat stable than C toxins (Table 2), and 90% reduc-

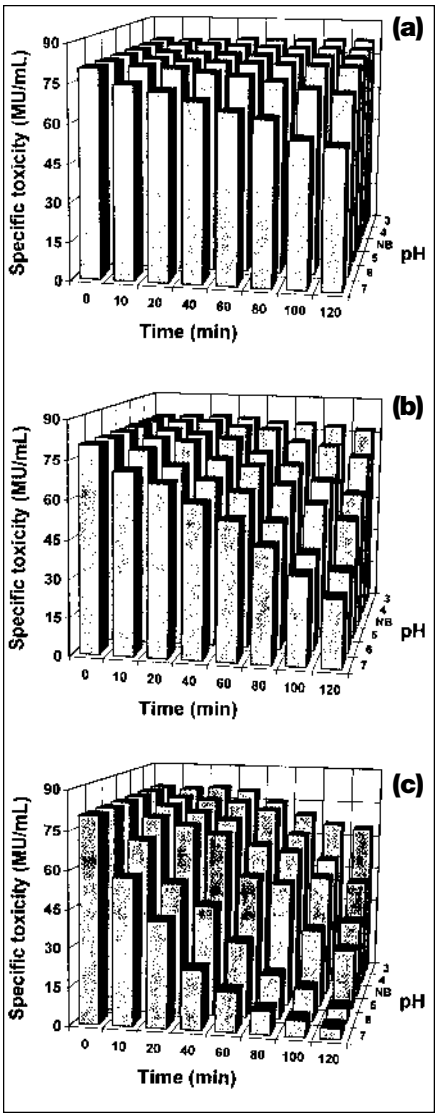

Fig. 6—(a) Variation of specific toxicities of the standard toxin mixture heated at 90 °C at different time and pH (b) Variation of specific toxicities of the standard toxin mixture heated at 110 °C at different time and pH (c) Variation of specific toxicities of the standard toxin mixture heated at 130 °C at different time and pH

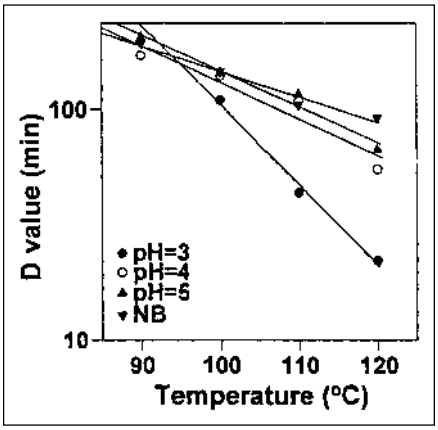

Fig. 7—TDT curves for C1/C2 toxins in the standard toxin mixture

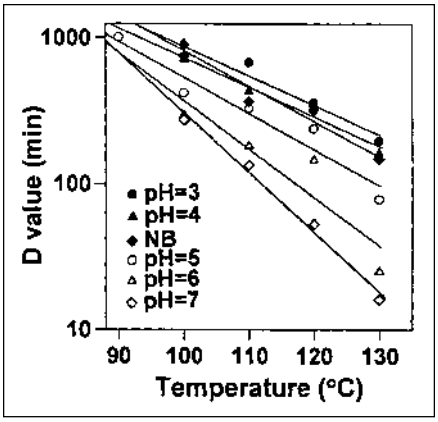

Fig. 8—TDT curves for GTX 1/4 toxins in the standard toxin mixture

tion of GTX 1/4 could be achieved by heating at 120 °C for 56.8 min at pH 7 or for 360 min at pH 3. D values for GTX 2/3 in the standard toxin mixture also decreased with increasing pH and temperature and ranged from 53.7 to 378 min (pH 7 to 3) at 120 °C. It is also interesting to note that STX had the highest D values, especially at low pH, suggesting that STX was the most heat-stable toxin examined. However, D values at 120 °C for NEO and STX varied from 89.7 to 380 min and 259 to 997 min, respectively, for the same pH range. The extremely high D values of STX clearly indicated the thermal resistance of STX, especially at low pH. TDT curves for the theoretical integrated specific toxicities calculated from HPLC data are shown in Fig. 9, and it is clear that D values decrease with increasing temperature and pH, with the values ranging from 117.6 to 842 min at 120 °C for the pH range of 7 to 3. At all temperatures, the lowest D values for all carbamate toxins were obtained at pH 7, whereas the highest values were obtained at pH 3.

z values were calculated as the negative slope of the "phantom" TDT (thermal death time) curves obtained by plotting D values at different temperatures on a logarithmic scale with the corresponding temperatures on a linear scale. z values indicate the temperature range necessary to bring about 10-fold change in the TDT or D value and may also vary according to the type food components and food-related microorganisms (Banwart 1989). The higher the z value, the more thermally stable the compounds are. z values calculated for the individual PSP toxins in the standard mixture are shown in Table 3. STX had the highest z values, indicating high thermal stability. However, the reaction kinetics of these toxins reported in the present study could have been different if the individual toxins were heated separately, since complicated concurrent oxidation and reduction reactions could result in the toxin interconversions when heated the toxin mixture, in addition to their possible degradation. Further studies on the structural analyses and toxicity of the products resulted by heating as well as the stoechiometry of the reactions are necessary to better understand the actual mechanism of reaction kinetics.

Table 1—Decimal reduction times (D, min) for C toxins (C1C2)

pH Temp.	3	4	5	6	7	NB
90 °C D=117.25	r^2 = 0.91 D = 171.9	r^2 = 0.78 D = 194.1	r^2 =0. 86 D = 187.4	r^2 = 0.91 D = 226.6	r^2 = 0.598 D = 206.8	r^2 = 0.77
100 °C D = 109.7	r^2 = 0.89 D = 140.1	r^2 = 0.85 D = 147.5	r^2 =0.96 D = 189.6	r^2 = 0.91 D =?	r^2 = 0.498 D = 144.7	r^2 = 0.77
110 °C D = 43.6	r^2 = 0.91 D = 108.8	r^2 = 0.96 D=104.9	r^2 = 0.96 D=374.19	r^2 = 0.69 D = ?	r^2 = 0.432 D=116.5	r^2 = 0.96
120 °C D = 22.3	r^2 = 0.95 D = 55.07	r^2 = 0.95 D = 92.3	r^2 =0.94 D = 296.2	r^2 = 0.66 D =?	r^2 = 0.05 D = 67.1	r^2 = 0.89

**r^2 values of the TDT curve are not significant ($P \leq 0.05$)
NB = control samples without buffer

Table 2—Decimal reduction times (D, min) for GTX 1/4

pH Temp.	3	4	5	6	7	NB
90 °C	**	**	r^2 =0. 41 D =1007.1	r^2 = 0.78 D = 385.9	r^2 = 0.88 D = 366.4	**
100 °C	r^2 = 0.86 D = 744.1	r^2 = 0.92 D = 712.1	r^2 =0.93 D = 419.4	r^2 = 0.97 D = 295.4	r^2 = 0.97 D = 273.6	r^2 = 0.43 D = 889.9
110 °C	r^2 = 0.89 D = 681.1	r^2 = 0.91 D = 431.4	r^2 = 0.94 D = 329.1	r^2 = 0.94 D = 182.9	r^2 = 0.92 D = 135.1	r^2 = 0.93 D = 368.7
120 °C	r^2 = 0.92 D = 360.3	r^2 = 0.94 D =351.9	r^2 =0.96 D = 239.4	r^2 = 0.98 D = 145.9	r^2 = 0.98 D = 56.9	r^2 = 0.79 D = 320.5
130 °C	r^2 = 0.94 D = 197.2	r^2 = 0.97 D = 162.4	r^2 = 0.91 D = 78.5	r^2 = 0.94 D = 25.3	r^2 = 0.99 D = 16.2	r^2 = 0.97 D = 148.7

** r^2 values for survivor curves are not significant ($P \leq 0.05$)
NB = control samples without buffer

Table 3—z values (C°) for standard PSP toxins

pH Toxin	3	4	5	6	7	NB
C1/2	r^2 = 0.91 z = 28.9	r^2 = 0.94 z = 62.7	r^2 =0.97 z = 89.5	**	**	r^2 =0.97 z = 63.9
GTX 1/4	r^2 = 0.93 z= 49.8	r^2 = 0.95 z= 49.6	r^2 = 0.93 z= 40.6	r^2 = 0.84 z= 30.3	r^2 = 0.99 z= 24.5	r^2 = 0.94 z= 41.6
GTX 2/3	**	r^2 = 0.87 z = 60.3	r^2 = 0.99 z = 39.8	r^2 = 0.98 z = 34.3	r^2 = 0.94 z = 27.9	r^2 = 0.94 z = 42.9
NEO	**	**	r^2 = 0.99 z= 56.1	r^2 = 0.99 z= 38.7	r^2 = 0.99 z= 35.2	r^2 = 0.99 z= 54.7
STX	**	**	r^2 = 0.98 z = 59.9	r^2 = 0.98 z = 38.9	r^2 = 0.98 z = 35.3	**
Specific Toxicity	**	r^2 = 0.97 z= 45.4	r^2 = 0.98 z= 44.9	r^2 = 0.99 z= 41.8	r^2 = 0.99 z= 38.5	r^2 = 0.98 z= 44.4

** r^2 values for TDT curves are not significant ($P \leq 0.05$)
NB = control samples without buffer

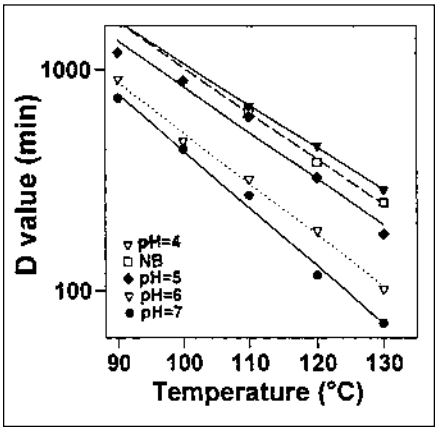

Fig. 9—TDT curves for specific toxicities of the standard toxin mixture

It is clear that pH, temperature, and heating time together, rather than individually, affect the degradation of any PSP component in a mixture in the absence of a matrix. Linear-regression models derived for the destruction of individual toxins could be improved by the addition of interaction term (pH x time), and the best-fitted models for the degradation of individual standard toxins as well as for the specific toxicity are shown in Table 4. The nature of the normal probability plot and the residual plot of each model were also checked, in addition to the F-ratio and p-value, to determine the best fit.

Conclusions

ALTHOUGH IT IS CLEAR THAT INDIVIDUAL toxins behave differently upon heating, the overall integrated toxicities for this particular buffered toxin mixture were reduced semi-logarithmically with time of heating as shown in Tables 1 to 3. One rea-

Table 4—Statistical models for the degradation of PSP toxins in the standard PSP toxin mixture

Model	R^2	F-ratio	P-value
GTX 2/3 = $-12.1 + 0.000001(t^3.T^3.pH^3) - 0.002(T)^3 - 0.872(pH^3) - 0.109(t.T.pH) + 16.4(t.pH) - 0.005(t^2.pH^2) + 34(T)$	94.6	442.5	0
GTX 1/4 = $-10.8 + 2.01(T) + 0.001(pH^2.t^2) + 1.67(t.pH) - 0.022(t.T.pH) - 0.068(pH)^3 - 0.00009(T)^3$	92.8	324.8	0
NEO = $-22.4 + 1.28(T) + 0.002(pH^2.t^2.T^2) + 1.76(t.pH) - 0.012(t.T.pH) - 0.052(pH)^3 - 0.0002(T)^3$	94.2	322.7	0
STX = $-10.2 + 0.000001(t^3.T^3.pH^3) + 0.12(t.pH) + 0.02(t^2) - 1.2(pH^2) + 10.4(T) - 0.0012(t.T.pH) - 0.0623(T^2) + 0.211(T.pH)$	98.4	526.4	0
C1/C2 = $-4.6 + 0.004(T^3) - 0.682(pH^3) - 0.224 (t.T.pH) + 6.2(t.pH) - 0.002(t^2.pH^2) + 22(T)$	96.6	492.1	0
Specific toxicity (MU/mL) = $14 + 0.609T + 0.711t + 25.7pH - 0.121 (T.pH) + 0.125(t.pH) - 0.00287(T.t.pH) - 0.00227(T.t) - 1.34(pH)^2 + 0.0000001(t.T.pH)^2$	95.2	507.1	0

[t = time (min), T = temperature (°C)]

son for this observation is perhaps that the original unheated mixture contained such a small portion of the N-sulfocarbamoyl toxins (C1, C2, B1) as compared to the carbamate toxins. It is likely that the kinetics of thermal destruction would have been far less favorable (higher D-values), and possibly non-1st order, had the mixture contained higher levels of N-sulfocarbamoyl toxins. It is because of the difference in the kinetics of thermal destruction among the various PSP toxins heated under different conditions that make it impossible to determine a generalized model to accurately predict the total integrated toxicities based upon time-temperature data.

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Authors are with the Department of Food Science and Technology, DalTech, Dalhousie University, P.O. Box 1000, Halifax, Nova Scotia, B3J 2X4. Direct correspondence to T.A. Gill (E-mail: Tom.Gill@dal.ca).