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THE FATE OF DOMOIC ACID IN DUNGENESS CRAB (*CANCER MAGISTER*) AS A FUNCTION OF PROCESSING

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ABSTRACT High levels (up to 90 μg/g) of domoic acid, a naturally occurring neurotoxic amino acid responsible for amnesic shellfish poisoning, were found in the viscera of raw Dungeness crabs during the 1991–1992 season. In studies reported here, Dungeness crabs were fed domoic acid contaminated razor clam meats for 6 or 9 days. Analyses of the raw crabs indicated that they rapidly accumulated domoic acid and that the toxin was confined to the viscera, principally in the hepatopancreas (22 μg/g). No domoic acid was detected in either body or leg meats of the raw crabs. When the whole crabs were cooked in either fresh or salted water, using a typical commercial cooking process, visceral domoic acid concentrations were reduced by 67–71%. After processing, the toxin was found outside of the visceral cavity in the body (1.9 μg/g) and leg (1.1 μg/g) meats. The majority of the domoic acid was extracted out and diluted into the cook water (0.4–0.8 μg/ml). The effect of storage conditions, typical of those used following processing and distribution, are also reported. Cooked crabs were held 1 day at 1°C and then analyzed; domoic acid was detected in the viscera and body meats. Portions of the body meat proximal to the viscera had higher domoic acid levels than those distal, but the toxin levels never exceeded 2.1 μg/g. This was also the case in crabs held for 6 days at 1°C and can be explained by simple diffusion. No domoic acid was found in leg meats of the 1 and 6 day storage samples. After storage for 90 days at −23°C, domoic acid was then found in very low concentrations outside of the viscera in the body (<0.8 μg/g) and leg (<0.4 μg/g) meats of the crabs. Our results indicated that the storage conditions had some affect on the toxin distribution throughout the cooked crabs, but had no affect on the total domoic acid burden in each Dungeness crab.

KEY WORDS: Domoic acid, amnesic shellfish poisoning (ASP), Dungeness crab, *Cancer magister*, razor clams, *Siliqua patula*

INTRODUCTION

Domoic acid (DA) is the naturally occurring neurotoxic amino acid responsible for amnesic shellfish poisoning (ASP) (Quilliam and Wright 1989, Todd 1993). This marine biotoxin causes gastrointestinal and neurological symptoms in humans within 24–48 hours of consumption, and symptoms range from simple nausea and vomiting to headache, confusion, permanent short-term memory loss, coma, and even death. Since 1987, studies in Canada and elsewhere have found that domoic acid can originate from phytoplankton, specifically the diatom genus *Pseudonitzschia*. To date, domoic acid has been found to be produced by at least 4 of the 21 species of *Pseudonitzschia* diatoms: *P. pungens forma multiseries* (Hasle) Hasle, *P. australis* Frenguelli, *P. delicatissima* (Cleve) Heiden, and *P. seriata* (Cleve) Peragello (Lundholm et al. 1994).

The first outbreak of domoic acid poisoning in the United States occurred in September of 1991. While no human illnesses similar to those reported in the 1987 Canadian outbreak occurred, the toxin caused more than 100 pelicans (*Pelecanus occidentalis* Linnaeus) and cormorants (*Phalacrocorax penicillatus* Brandt) to become ill or die. The seabirds had been feeding on anchovies (*Engraulis mordax* Girard), which had grazed on a bloom of *P. australis* (Work et al. 1993). Samples of these anchovies were analyzed in our laboratory and found to contain up to 485 μg DA/g viscera (Wekell et al. 1994a).

Shortly thereafter, domoic acid was discovered throughout the Pacific coastal states of Oregon and Washington in October of 1991. An extensive survey of coastal marine life was performed, and the toxin was found in anchovies, blue mussels (*Mytilus edulis* L.), razor clams (*Siliqua patula* Dixon), and Dungeness crabs (*Cancer magister* Dana). During the toxin's peak in Washington, our laboratory reported up to 230 μg DA/g meat tissue (average = 106 μg/g; N = 36) in razor clams, and up to 90 μg DA/g raw viscera (average = 15 μg/g; N = 87) in Dungeness crabs. The source of domoic acid in Washington and Oregon was never confirmed; however, *P. australis*, *P. pungens f. multiseries*, and *P. pseudodelicatissima* (Hasle) Hasle have been identified in waters of the Pacific Northwest (Horner 1993, pers. comm.). Human illnesses were avoided because of the timely action of local, state, and federal agencies identifying the threat and closing the affected fisheries until they were considered safe (i.e., below 20 μg DA/g). During 1992, the legal limit for crab viscera was raised to 30 μg DA/g (U.S. FDA 1993). These closures and subsequent restrictions have had significant financial impacts on both the fisheries and the supporting communities.

The Dungeness crab industry is an important component of the economies of the Pacific coastal states. In Washington, Oregon, and California there are over 1,200 crab fisheries. This industry lands an average of $32 million pounds of crab per season, results in an average annual post-processing value of $112 million, and contributes significantly to employment in otherwise economically depressed areas (Didier 1995).

It was because knowledge concerning domoic acid production and movement through the marine food web was extremely limited, that state and federal risk managers had little choice but to implement total closures of the Dungeness crab and razor clam

fisheries in these states in December 1991. In order to help re-open these fisheries, our laboratory participated in surveys and lot analyses of Dungeness crab from Oregon and Washington, and samplings of razor clams from the Washington coast. This data provided a model that indicated the breadth and depth of domoic acid contamination in both Dungeness crab and razor clams.

In our analyses, we examined only raw or un-cooked molluscan shellfish and crabs (Wekell et al. 1994a, b). In these investigations, we found that domoic acid was confined to the digestive system in the live or uncooked crabs. We also observed that levels of domoic acid in cooked crabs were considerably lower than in raw crab samples from the same area. It appeared that processing the crabs reduced domoic acid levels. In order to test this hypothesis, we conducted several studies on Dungeness crab contaminated with domoic acid.

EXPERIMENTAL APPROACH

The use of naturally contaminated Dungeness crabs in these studies was not possible since, at the time (spring and summer of 1994), domoic acid levels in crab had declined to very low levels (i.e., typically 0–5 µg DA/g raw viscera). In addition, the variability from animal to animal was extremely high (Wekell et al. 1994a). This high variance would have required very large numbers of animals to detect statistical differences expected in such a study. Previous work in our laboratory indicated that crabs could be manually fed razor clams naturally contaminated with domoic acid. The resulting variation of toxin levels between animals in the lab was significantly smaller than that found in the natural state. In these studies it was possible to follow on a daily basis the uptake and depuration of domoic acid by the Dungeness crabs. (Lund, in prep.) This presented an opportunity to investigate crabs held under laboratory conditions and fed a portion-controlled diet containing known concentrations of domoic acid.

In a pilot study, we fed Dungeness crabs toxic razor clams and sampled a subset of the crabs daily. The raw crabs were dissected into sections (individual organs of the viscera, body, and leg meats) and all parts were analyzed for domoic acid. From this pilot study, we determined how much of the clam meat the crab would eat per feeding, as well as the rate and distribution of toxin uptake. The results confirmed that in the raw Dungeness crabs, no domoic acid is detected outside of the viscera; and the toxin remains exclusively in the hepatopancreas and the digestive track (data not shown).

After the pilot study, 2 experiments were performed to determine whether domoic acid migrates from the viscera of Dungeness crabs into the cook water and/or into the meat as a result of cooking and post-processing conditions. In the first experiment, the goal was to investigate the fate of domoic acid as a result of cooking the Dungeness crabs in either fresh or salt (3% NaCl) water. In the second experiment, the objective was to investigate the fate of domoic acid in cooked crab as a result of post-processing storage conditions.

MATERIALS AND METHODS

Reagents

Methanol (MeOH) and acetonitrile (MeCN) were HPLC-grade (Baxter Healthcare Corp., Burdick and Jackson Division, Muskegon, MI 49442). The trifluoroacetic acid (TFA) was obtained from Sigma (Sigma Chemical Company, St. Louis, MO 63178). The sodium chloride (NaCl) and all other reagents were analytical grade. All solutions were prepared with distilled deionized water (Milli-Q, Millipore, Bedford, MA 01730).

Standards

Domoic acid was obtained as a certified standard (DACS-1, Canadian National Research Council, Institute of Marine Biosciences, 1411 Oxford Street, Halifax, N.S., Canada B3B 3Z1), and as a 90% pure reagent from Sigma. The DACS-1 was used to prepare a 2.00 µg DA/ml 10% aqueous MeCN instrument calibration standard, and the 90% pure reagent was used to prepare a 20 µg DA/ml 50% aqueous MeOH quality assurance sample. The standards were stored in the refrigerator when not in use.

Razor Clams

Clean, non-toxic razor clams were purchased from a local supermarket. The clams were obtained from a supplier in Alaska and were analyzed for domoic acid content in our laboratory. Levels of domoic acid in these clams were non-detectable. The non-toxin bearing clams were fed to the crabs during the first 24–48 hours of captivity to permit acclimatization to the laboratory environment and feeding regime.

The toxin source for the feed in these experiments were raw, frozen, canned razor clams. The clams had been harvested during the 1991 domoic acid incident along the west coast and were embargoed because of the high domoic acid content. We were permitted to purchase the clams from a commercial supplier in Oregon for experimental purposes. The frozen cans were sampled by coring, and analyzed. The toxic clam cores averaged 40.3 µg DA/g tissue (range: 36.3–50.2 µg/g; N = 4 cans) for Experiment 1; and 57.1 µg DA/g tissue (range: 48.1–62.2 µg/g; N = 10 cans) for Experiment 2. Daily feed composites samples were also collected during the experiment and analyzed; these samples averaged 27.6 µg DA/g tissue (range: 25.6–31.4 µg/g; N = 6) for Experiment 1 and 45.5 µg DA/g tissue (range: 34.6–53.8 µg/g; N = 9) for Experiment 2. The difference in DA concentration of the feed core samples and the daily feed samples was due to the loss of DA in the clam drip or thaw liquid.

Dungeness Crab

To insure our test animals were free of domoic acid, we acquired live Dungeness crabs caught in the Pacific Ocean off the Canadian and Alaskan coasts in areas where the toxin has not been detected. We purchased 56 crabs for Experiment 1, and 73 for Experiment 2 from a company in Washington state. A subset of 6 to 12 crabs were randomly selected prior to each experiment and individually analyzed for domoic acid to confirm the absence of toxin.

Conditions

The Dungeness crabs were transported from the purchasing site in chilled coolers and then held in large glass or fiberglass tanks at our Mukilteo, WA facility. The tanks were filled with sand and gravel filtered Puget Sound sea water (12.2–13.3°C) and continuously refreshed with a flow-through system at a rate of 4–6 L/min. Each tank held 8 to 9 crabs.

EXPERIMENTAL OUTLINE

Crab Feeding

The crabs were labeled with a number and acclimated to their new environments for 24–48 hours; during this time they were fed

a small amount of clean razor clam meat (≤12 g). This initial feeding and time interval allowed for natural fatalities to occur before the experiment began, and permitted identification of obvious and possible poor eaters.

The crabs were then hand fed 10 to 18 g of toxic razor clams using long-armed utility claws for 6 (Experiment 1) and 9 (Experiment 2) days, excluding weekends. Experiments 1 and 2 were carried out over a period of 9 and 13 days, respectively. The goal of these feedings was to obtain crabs with viscera domoic acid contents above 20 µg/g. The amount of clam meat and number of days to feed were determined accordingly. During the experiments each group of crab was fed an average of 30.7 mg DA (Experiment #1) and 55.3 mg DA (Experiment #2).

As each experimental study proceeded, dead or dying crabs, and poor or non-eating crabs were removed from the tanks. When the feeding was complete, the crabs were randomly divided into experimental groups; for each study, all groups contained 12 crabs. The crabs were removed from the tanks, packed in coolers and transported within 1 hour to our Seattle, WA facility. Upon arrival at our laboratory, the crabs were immediately sacrificed and processed (either as raw samples or utilized in the experimental cooking and/or storage studies).

Experiment 1: Fresh vs. Salt Water Cooking

The object of this study was to determine if cooking in salt water (3% NaCl) or fresh (non-salted) water had an effect on the resulting domoic acid content in the crab. In the study, the crabs were divided into 3 groups: raw control crabs, fresh water cooked crabs, and salt water cooked crabs. The cooked crabs were boiled for 20 minutes in 18 gallons of either fresh or salt water, using a steam-jacketed stainless steel commercial cooker. After cooking, the crabs were rinsed briefly with cold water, drained, and allowed to cool. Caution was taken to place each crab in the same orientation during the cooking and cooling processes. Once cool, a composite of the drip was collected from each cooked group. All the crabs were then dissected into viscera (hepatopancreas only), body and leg (merus) meats. The samples were refrigerated (1°C) less than 24 hours and analyzed.

Experiment 2: Post-Processing Storage Conditions

The object of this study was to determine the effects, if any, of storage on domoic acid content of cooked crab. In this experiment, the crabs were divided into 4 groups: raw control crabs, salt water cooked crabs held 1 and 6 days at 1°C, and salt water cooked crabs held 90 days at −23°C. Three groups of crabs were boiled for 20 minutes in 27 gallons of salt water. Again, the crabs were cooked, rinsed, drained, and allowed to cool with special attention paid to crab orientation. Each crab was then individually bagged and placed vertically (posterior up) on large trays. The groups were stored for 1 day at 1°C, 6 days at 1°C, or 90 days at −23°C before sampling. The drip was collected, if available, and the crabs were dissected into viscera (all of the gut soft tissues, excluding gills and heart), body meat proximal and distal to the viscera (the meat between the visceral cavity and the leg knuckles was divided into two equal parts), and the leg meat (merus and knuckle meat from the first walking leg). All samples were analyzed immediately after the allotted storage time had expired.

Sample Preparation

All tissues collected in the studies were homogenized using a common household blender. To ensure thorough homogenization, the meat samples (body or leg) were mixed 1:1 (wt:wt) with distilled water prior to blending. Allowances were made for this added water in the final calculations of domoic acid concentration. Visceral tissues were homogenized without the addition of water.

Domoic Acid Analysis

All samples were analyzed by the methanol extraction method of Quilliam et al. (1989, 1991) with modifications to the solid phase extraction (SPE) clean-up step (Hatfield et al. 1994). All analyses were performed on a Hewlett-Packard 1090 High Performance Liquid Chromatograph (HPLC) equipped with a Vydac 201TP column (Reversed phase C_{18}, 2.1 mm × 25 cm, Separations Group, Hesperia, CA 92345), and a diode array detector set at 242 nm with a 10 nm bandwidth. The domoic acid was chromatographed isocratically at 40°C with H_2O/MeCN/TFA (90/10/0.1) (v/v/v) at a flow rate of 0.300 ml/min. Sample injections of 20 µl. were used, and the domoic acid retention times were between 7 and 9 minutes. A 2.00 µg/ml DACS-1 standard was included before, after, and within sets of samples for calibration and quantitation. A 20 µg DA/ml. quality assurance sample was always included to assure that full (>90%) recovery of the domoic acid was obtained from the SPE step.

RESULTS AND DISCUSSION

Experiment 1: Fresh vs. Salt Water Cooking

The effect of salt in the cook water during processing of the Dungeness crab was examined. After the 6 feedings with contaminated razor clams, the raw control group indicated the crabs attained an average of 22.1 µg DA/g hepatopancreas. Based on the mass balance analysis of the raw crab viscera, approximately 42.3% of the total domoic acid feed dose was retained by the Dungeness crabs. After processing, the fresh and salt water cooked crabs yielded domoic acid concentrations of 6.39 and 6.43 µg/g, respectively (Table 1). When considering the original DA concentration in µg/g, there was a 71.1% reduction in domoic acid in the hepatopancreas after cooking the crabs in fresh water, and a 70.9% reduction after cooking the crabs in salt water. Looking at the absolute value of micrograms of DA content in the hepatopancreas, there was an average loss of 78.8% in the fresh water and 79.7% in the salt water cooked crabs. Therefore, the addition of salt had no effect on the resulting domoic acid concentration in the crabs.

Domoic acid is a highly water soluble, low molecular weight amino acid (Falk et al. 1991). Once the physiological and biological barriers in the crab are disrupted and their integrity compromised during the cooking process, the toxin would be expected to migrate throughout the crab and into the cook water. Direct analysis of the cook waters by HPLC indicated 0.81 µg DA/ml for the fresh cook water and 0.72 µg DA/ml for the salt water. This would partially explain the large reduction in the visceral domoic acid content; although we can not eliminate the possibility of chemical alteration of the DA (i.e., loss of the molecule's UV chromophore).

Experiment 2: Post-Processing Storage Conditions

The effect of post-processing storage conditions on domoic acid concentration and distribution in the Dungeness crab was examined. Based on the raw control group (Figure 1), the crabs in this study attained an average domoic acid concentration of 22.4

TABLE 1.

Experiment #1: Dungeness crab weight (g) and domoic acid (DA) concentration (μg/g).

Group	Statistics	HP Wt. (g)	HP DA conc. (μg/g)	BR or BC Wt. (g)	BR or BC DA conc. (μg/g)	LC Composite Wt. (g)	LC Composite DA conc. (μg/g)
Blank (raw, non-fed)	Ave:	NA	0	NA	0	NA	0
Raw	Ave:	49.41	**22.08**	NA	0	NA	0
	Std Dev:	7.23	7.89		0		
	%CV:	14.63	35.72				
	Low:	35.90	6.46		0		
	High	61.94	33.00		0		
Fresh water cooked	Ave:	36.05	**6.39**	63.09	**1.88**	126.5	**1.10**
	Std Dev:	8.16	1.68	11.94	0.59		
	%CV:	22.64	26.34	18.92	31.53		
	Low:	25.7	3.57	49.34	1.03		
	High	51.77	9.23	83.54	3.12		
Salt water cooked	Ave:	34.37	**6.43**	66.82	**1.86**	120.9	**1.11**
	Std Dev:	6.55	2.00	8.27	0.42		
	%CV:	19.06	31.05	12.38	22.40		
	Low:	22.83	4.54	53.36	1.34		
	High	46.2	11.24	80.80	2.80		

NA = Not Available; HP = Hepatopancreas; BC = Body meat cooked; BR = Body meat raw; LC = Leg meat cooked (composite of the menis meat of the first walking leg from each of the 12 crab); Ave = average or arithmetic mean; Std Dev = standard deviation about the mean; %CV = percent coefficient of variation (standard deviation divided by the mean times 100).

μg DA/g viscera and retained approximately 47.3% of the total domoic acid feed dose. No domoic acid was found outside of the viscera in the raw crabs. Again, there was a reduction (67%) in viscera domoic acid μg/g concentrations after cooking (Table 2). Domoic acid burden, total μg of domoic acid per crab, was reduced by nearly 68%. The toxin lost was detected in the cook water at a concentration of 0.4–0.6 μg DA/ml, and in the drip at 1.25 μg DA/ml. The crab meat sections also had detectable amounts of domoic acid (Figure 1).

The length of storage after cooking had little effect on the absolute domoic acid concentration in the crabs. When all 3 storage conditions were compared, no significant differences ($p < 0.05$) were found in the domoic acid concentration in the viscera and the distal body meats. There were, however, differences detected between the toxin levels in the proximal body and leg meats in the 3 groups. Domoic acid was only detected in the leg meats from the samples frozen at −23°C for 90 days.

The freezing and thawing of the crabs had an effect on the distribution of the domoic acid within the crab. With the crabs held for 1 or 6 days at 1°C, there was a relationship between the domoic acid concentration in the meat and its proximity to the viscera. The closer the body meat was to the viscera, the higher the domoic acid concentration. These observations can be explained by assuming a simple diffusion model. This did not seem to be the case with the 90-day frozen samples; the toxin appeared randomly distributed throughout the crab. Regardless of the domoic acid distribution within the crabs, the resulting absolute domoic acid content in the

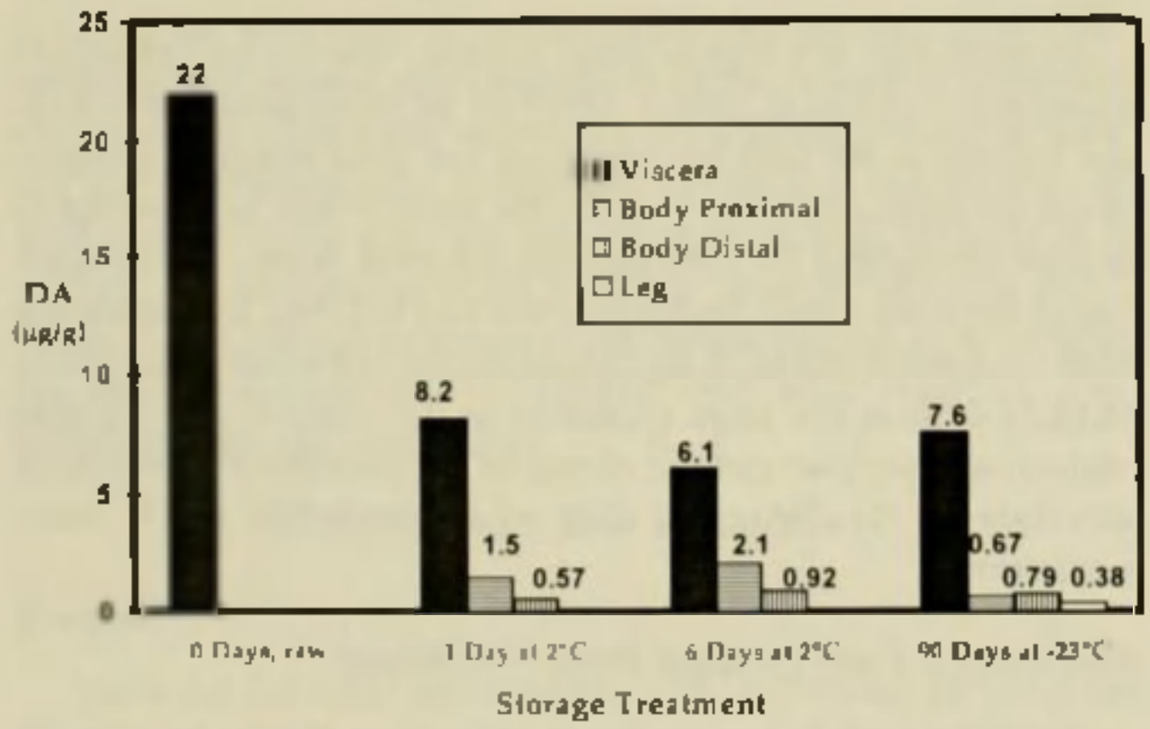

Figure 1. Experiment #2: Domoic acid (DA) distribution and concentration (μg/g) in cooked Dungeness crab resulting from different post-processing storage conditions (length of time held after cooking and temperature); referenced to raw Dungeness crab analyzed immediately after sampling.

TABLE 2.

Experiment #2: Average domoic acid (DA) content (μg) and concentration (μg/g) in raw and cooked Dungeness crab viscera.

Group (N = 12)	Total Viscera DA Content (μg)	Average Absolute % Loss (μg)	Average Viscera DA Conc. (μg/g)	Average Relative % Loss (μg/g)
RAW CRAB				
No storage	**26,194.4**		**22.38**	
COOKED CRAB				
1 day at 1°C	9,176.0	65.0	8.18	63.4
6 days at 1°C	8,016.7	69.4	6.11	72.7
90 days at −23°C	8,102.4	69.1	7.64	65.9
Ave:	**8431.7**	**67.8**	**7.31**	**67.3**

TABLE 3.

Experiment #2: Estimated absolute domoic acid (DA) content (µg) in cooked Dungeness crab stored under three different treatment conditions.

Cooked Group (N = 12)	Total DA Content (µg)
1 day at 1°C	10,502
6 days at 1°C	10,122
90 days at −23°C	10,006
Ave:	**10,210**
Std Dev:	**259**
% CV:	**2.5**

cooked Dungeness crabs was essentially the same for all 3 treatment groups (Table 3).

CONCLUSIONS

These studies indicated that the concentration of domoic acid taken up by the Dungeness crabs during feedings was decreased by 67–71% during the cook processing. The bulk of the domoic acid was extracted from the crabs and diluted into the cook water and drip. After cooking, the major portion of the domoic acid remaining in the crabs was found in the viscera. Only very low levels of the toxin were detected in the body or leg meats. Storage time and temperature did not affect the overall burden of the toxin in the crab: although freezing/thawing did change the distribution of the domoic acid within the crab.

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