The 4th International Conference on Agriculture and Agro-Industry
ICAAI2022
Transforming Value Chains for Global Security
Mae Fah Luang University, Chiang Rai, Thailand
November 10 -11, 2022

Journal of Food Science and Agricultural Technology

International peer-reviewed scientific online journal Published online: http://rs.mfu.ac.th/ojs/index.php/jfat

Original Research Article

Assessment of Paralytic Shellfish Poisoning Toxins in Green Mussel *(Perna virisdis)* of Street Food Grilled Dish in Bangkok: A Food Safety Concern

P. Nochit *, W. Kulsawat, R. Samran and J. Khunsamut

Thailand Institute of Nuclear Technology (Public Organization), Ongkharak district, Nakhon-Nayok 26120 Thailand

ARTICLE INFO

Article history:
Received 11 November 2022
Received in revised form 22 July 2023
Accepted 31 July 2023

Keywords:
Green mussel
Paralytic shellfish poisoning toxin
Radioligand receptor binding assay
Street food grilled dish
Thailand

ABSTRACT

The notable street food grilled dish in Bangkok is known for its unique flavors and tasty aroma, easily accessible, inexpensive and nutritious. The consumption of street foods has become a public health concern. A major source of contribution to health hazard risk is raw materials. Paralytic shellfish poisoning toxins (PSTs) or saxitoxin (STX) can cause severe neurological effects. This illness caused by intaking PSTs contaminated molluscs. The preliminary study aimed to assess PSTs in fresh green mussels from street food grilled dishes in Talad Phu and Ram Intra subdistrict in Bangkok during 2021. Seventy composite samples of green mussels (a pool of 20 individuals for each) were randomly collected from street vendors and determined PST using a radioligand receptor binding assay. The PSTs in the examined samples ranged from 0.285 to 5.477 with the average of 2.619 ± 0.995 µg STX eq/100 g meat. The results revealed that all of the examined green mussels of street food grilled dishes were of satisfactory quality due to toxins levels not exceeding a safe level of 80 µg STX eq/100 g meat. It indicates that there is no health risk for green mussel consumers of street food grilled dishes in the examined vendors.

© 2023 School of Agro-Industry Mae Fah Luang University. All rights reserved.

INTRODUCTION

Street foods are defined as ready-to-eat food that prepared or sold to the customers in the street, sidewalk and other public places. Street foods can be consumed without further processing or cook. The consumption of street food is not only an economical but also practical for the people with all ages. The consumers denoted a variety of careers with various socioeconomic classes included students, travelers and tourists. It was recognized that street food grilled dishes in Bangkok are tastiness, easily accessible, varieties of shellfishes offer, inexpensive and nutritious. However, the safety concerns of these foods are increasing worldwide. It was documented that the risk of food borne sicknesses are directly related to the quality of raw materials (WHO, 2010).

Shellfish are a filter feeder organism, can live in polluted water, and are able to store pollutants in their tissue without apparently any harm; therefore, they are a high-risk food. The pollutants include chemicals, radionuclides, biotoxins, pathogenic bacteria, and viruses (Berdalet, et al., 2015). Paralytic shellfish poisoning toxins (PSTs) or saxitoxin (STX) is a naturally occurring toxins produced by marine dinoflagellates. STX affects many marine species; however, bivalve molluscs such as mussel, clams, and oyster can resist. These shellfishes filter large volumes of water and can accumulate STX in their tissues which can retain in a long period of time. (Blanco, et al., 2022; Botelho et al., 2019).

STX is a water soluble, heat, freeze, and acid persistent. The same as chemicals and radionuclides, STX cannot be destroyed or removed by cooking, heating, and freezing. In addition, STX may be released during boiling, becoming more concentrated in the broth. It is a neurotoxin that can cause severe and life-threatening

* *Corresponding author*
E-mail address: phatchada@tint.or.th

ISSN: 2408-1736

neurological effects. The symptoms include tingling, numbness, burning of the throat, tongue and lips, diarrhea, vomiting, nausea and diaphoresis or excessive sweating. This condition can be fatal if respiratory muscles are paralyzed (Van Dolah, 2000; Etheridge, 2010). PSTs do not have distinctive odors or taste; they can be detected only through specialized laboratory testing. Method for detection PSTs in shellfishes includes mouse bioassay, ELISA, HPLC and radioligand receptor binding assay (r-RBA). Previous studies documented that application of r-RBA method provided an early warning of PSTs in shellfish which contributed to human health and environment mitigation. Instrument used by the r-RBA were less expensive compared to other techniques; it replaced used of living animals, rapidly, and high sample throughput. The r-RBA method is a very specific and accurate activity-based technique for the detection of PSTs (Ruberu et al., 2012, 2018; IAEA, 2013). The r-RBA can analyse samples with a wide range of PSTs level using an appropriate dilution factor. (Powell and Doucette, 1999; IAEA, 2013).

Several studies reported that different bivalves have different capacities to accumulate STX levels. Mafra, et al. (2010) revealed that in a laboratory experiment, oyster *C. virginica* has relatively low capacity to accumulate biotoxin than mussel *M. edulis* which due to feeding physiology. In agreement, Kacem et al., (2015) reported the toxicities of STX were 20–70 times greater in mussels *M. galloprovincialis* than in oysters *C. gigas*, also the mussel retained its toxicity for slightly and longer period than oyster. In Alaska, the mussel, *Mytilus edulis*, can accumulate in excess of 20,000 µg of STX per 100 g of tissue, an extremely dangerous level. In the Kodiak area during the summer of 1993, one death and several illnesses were attributed to mussels containing 19,600 µg of STX per 100 g of tissue. The extreme toxicity of mussels is due primarily to their high tolerance and insensitivity to STXs which enables them to continued feeding on toxic algae (Kacem et al., 2015). Therefore, mussel is suitable for an indicator species.

Although the street food will become more common in the future. In Thailand, there is no available information on the quality and safety of street-vended food concerning the incidence of PSTs. The present study was carried out to assess the PSTs of the street food grilled dishes in two subdistricts, Talad Phu and Ram Intra of Bangkok, Thailand. The PSTs determination method applied in this study was r-RBA of tritiated saxitoxin [^{3}H-STX] using microplate scintillation technology. This r-RBA method is capable to analyse qualitatively and quantitatively PSTs (IAEA. 2013).

MATERIALS AND METHODS

Chemicals and reagents

The required chemicals were consisting of 50 µCi of ^{3}H-STX in methanol with a specific activity 20.0 Ci/mmol, a reference standard consisting of STX dihydrochloride at 268.8 µM (100 µg/mLin 20% ethanol-water at pH 3.5) and receptors prepared from porcine membrane homogenates including an Optiphase Supermix scintillant. A buffer solution of pH 7.4 was prepared with morpholino propane sulfonic acid (MOPS) and aqueous choline chloride, adjusted to pH 7.4 with 3M NaOH (stored at 4 °C for its use within a period not exceeding one month).

Syringe filter (0.45 µm), 96-well MultiScreen plates and a micro beta scintillation counter are also needed in the assay.

Collection of samples

Green mussel samples were achieved by random sampling from street vendors at Talad Phu and Ram Intra subdistrict in 2021. Mussel collections were performed monthly on two occasions, dry season (Jan-Mar) and wet season (Jun-Aug). However, resampling would be required, if STX had been found at levels exceeding the safety limits. A composite sampling technique was conducted in this study. For this purpose, 10 individual green mussels were sampled twice a month as a representative for each vendor. Then the 20 mussel specimens (monthly samples per vendor) were combined in a single sample for analysis. Sample was kept in separate sealed plastic bag, frozen, and submitted to the testing laboratory. A total of 70 mussel samples were analysed for PSTs using r-RBA method. The appearance of green mussel, major raw materials, was also observed using sensory characteristics, such as odor (no foul smell) and color (freshness) to avoid bias. Information on sample collection was shown in Table 1.

Table 1. Information on sample collection.

No.	Subdistrict	Jan-Mar Sample/vendor	Jun- Aug Sample/vendor
1	Talad Phu	18	17
2	Ram Intra	18	17

Preparation of green mussel extract

Samples were kept in freezer prior to transport to the laboratory where their shells were removed and the whole flesh tissues were pooled and drained for 2 min. Flesh samples were homogenized with a blender and an aliquot of 20 g was extracted in an equal volume of 0.1 M HCl. The extract was heated in boiling water using water bath for 5 min, cooled to room temperature and adjusted pH to a value of 3 - 4. The extract was centrifuged (3000 g for 5 min) and filtered with a syringe (0.45 µm). After that, the supernatant was directly stored at -80 °C prior to toxin analysis.

Determination of PST using r-RBA

The r-RBA was used for the analysis of PSTs in the mussel extract. This assay is a directly competitive binding between PSTs in the mussel tissue and a radio-labelled STX (^{3}H-STX) for receptor sites in a porcine membrane homogenate, (Van Dolah et al., 1994, 2009, 2012; Doucette et al., 1997; Powell and Doucette, 1999; IAEA, 2013; Dechraoui Bottein and Clausing, 2017).

The r-RBA is carried out in 96-well MultiScreen plates (Merck Millipore, part # MS FBN6 B50) in a final volume of 210 µL per well. The unknown sample in the separate dilutions of 10, 20 and 50 was assayed in triplicate. To each well was added 35 µL of buffer, 35 µL of unknown sample, 35 µL of ^{3}H-STX, and 105 µL of a 1:9 diluted porcine membrane homogenate, vortex. covered the plate and incubated for 1 h at 4 °C to allow binding to reach equilibrium. After incubation, plate was immediately filtered using a MultiScreen vacuum manifold system (EMD Millipore, part # MSVMHTS00), and followed by two washes with 200 µL of ice-cold buffer to remove unbound toxin. A volume of 50 µL of the scintillant (Supermix PerkinElmer) was added to each well, the top of the plate sealed with adhesive tape and was incubated in darkness at room temperature for 2 h. The radioactivity of bound ^{3}H-STX was counted based on 3 min counting time per well in a micro beta scintillation counter (Wallac® TriLux 1450 MicroBeta counter).

Preparation of a standard curve

A calibration curve was generated using a set of serial dilution of unlabelled STX standard range of 10^{-6} to 10^{-10} M.

Data analysis

For each sample concentration, non-specific binding was subtracted from total binding to give specific binding. Data was fitted using a four-parameter logistic fit, a sigmoidal dose-response curve with variable slope. The binding of ^{3}H-STX is reduced in the competition of unlabelled STX as shown in Figure 1 (IAEA, 2013). However, curve fitting was performed when the relative standard deviation (RSD) of the triplicate data of the toxin standards was verified to be below 30%. The quantity of STX equivalents to the undiluted sample per 100 g tissues was eventually calculated.

GraphPad Prism (GraphPad Software, Inc., La Jolla, California, USA) was used to generate standard curves and to perform data analysis**.** One-way ANOVA was used to test for difference between seasonal variations (dry and wet season). Statistical significance was designed as being at the level of $p < 0.05$ (Van Dolah, 2012).

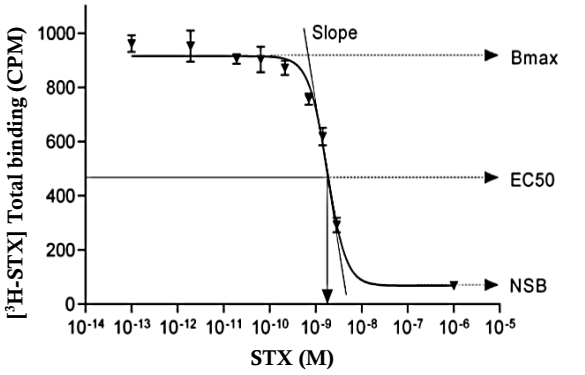

Figure 1. Competition of unlabelled toxin (STX) with the binding of radioligand labelled (^{3}H-STX) to the receptor (Bmax is a maximum binding of ^{3}H-STX without unlabelled STX; EC50 is the effective concentration at 50% and NSB is non-specific binding of the saturated unlabelled STX)

RESULTS AND DISCUSSION

Over 70 assays performing, it was found that the RSD of the triplicate values for each green mussel sample dilution was below 30% (average 12%). Also, the internal QC for each plate was within ± 30% of the known concentration. Therefore, the r-RBA for PSTs in this study was acceptance as defined in the AOAC validation (Van Dolah et al., 1994; 2000; 2012).

It should be noted that all raw materials of street food grilled dish of both Talad Phu and Ram Intra subdistrict were purchased from the public market. Vendors also had their own regular source from whom they purchase raw materials at a discounted price, especially if purchased in bulk. Vendors in this study declared that green mussels sold were all originated from domestic mussel farm located in Samut Sakhon, so called a native mussel. Although there were not any available native green mussels sold in the market, the imported mussels were not taken to replace the native ones (Private communication, 2021).

Results shown in Table 2 are mean values of 3 trials. The lower limit of detection was 2.25 µg STX eq/100 g meat. PSTs in the examined samples ranged from 0.285 to 5.477 with the average of 2.619 ± 0.995 µg STX eq/100 g meat.

The results of both Talad Phu and Ram Intra subdistrict shown that the level of PSTs in street food grilled dish were very similar and not statistically different. The levels of STX toxicity in µg STX eq/100 g meat differed insignificantly ($p > 0.05$) according to the dry and wet season. It was indicated that PSTs in the observed specimens did not reveal absence, but rather indicated that PSTs might still be presented in the samples. However, the values were below the permissible legal limit of 80 µg STX eq/100 g meat (IAEA, 2013). The sampling size of 20 individual mussel was reasonably adequate as variation in STX levels was not large. It was assumed that resampling was unnecessary due to the aim of this study was to spot check the quality of mussel raw material throughout street food vendors at Talad Phu and Ram Intra subdistrict.

These finding results indicated that there was no health risk for green mussel consumers of street food grilled dishes in the examined vendors.

Table 2. PSTs of green mussel sampling from street food grilled dish of two subdistricts in Bangkok (µg STX eq/100 g meat).

No.	Talad Phu Jan-Mar 2021	Ram Intra Jan-Mar 2021	No.	Talad Phu Jun-Aug 2021	Ram Jun-Aug 2021
1	0.873	2.299	19	2.718	2.976
2	1.011	1.577	20	2.614	2.722
3	0.914	1.126	21	2.094	3.669
4	0.989	2.248	22	1.235	3.463
5	2.216	1.259	23	2.581	3.058
6	2.346	1.235	24	3.334	4.892
7	2.323	1.162	25	3.267	3.924
8	0.285	1.583	26	3.117	3.458
9	2.362	2.708	27	1.018	2.884
11	2.414	3.032	28	0.855	3.012
12	3.721	3.252	29	2.346	2.556
13	3.168	3.369	30	2.362	3.174
14	3.214	3.405	31	3.587	3.429
15	3.599	3.599	32	3.749	5.477
16	3.193	4.132	33	2.898	2.619
17	2.848	2.245	34	3.225	2.539
18	2.221	2.596	35	2.611	2.118

CONCLUSION

It is well recognized that street food grilled dishes in Bangkok are popular among local people and tourists. Major raw materials are important source of biological and toxicological risks. One of the human health threatening has been due to consumption of shellfishes that contaminated with PSTs in the level of exceeding permissible limit. Surprisingly, there is an extremely scarcity of data to prevent this threat pose to customers, despite the street food will become more common in the future.

In this present study, the comparative PSTs accumulated in the green mussel (*Perna viridis)* were investigated using RBA of tritiated saxitoxin [^{3}H-STX] in microplate format. The finding results revealed the concentrations of PSTs in the examined green mussels were much lower than the acceptance limit which indicated that the examined green mussel available on street food grilled dish in Talad Phu and Ram Intra subdistrict were safe for consumers. Although raw materials of street food grilled dish in Bangkok are consisted of cockle, clam, mussel and scallop. This study only emphasizes on green mussel of street food. Further investigations on street food

grilled dishes of different species and in more subdistricts of Bangkok including the regular monitoring program are important to ensure safe for consumption. The reliable data about PSTs in green mussels from street vendors has assisted in improving the safety of street food grilled dishes. Information of contaminated PSTs in raw materials can be integrated with other toxicological hazards to implement preventive measures. The street food safety program needs to be developed to certify that all shellfish sold are safe for consumption.

LIMITATION OF THE STUDY

Although the presented study is the first of this kind in Thailand, it is some limitations. The authors recognise that the sample size was limited and the results should be interpreted as preliminary. Due to these limitations, further studies are needed.

ACKNOWLEDGEMENTS

The authors appreciate the anonymous reviewers of the critical review and the editorial staff of the Journal of Food Science and Agricultural Technology for assistance in the revision and production of this manuscript.

Special thanks to Asst. Prof. Dr. Saowapa Chaiwong, Chair of Academic Committee ICAAI2022 and her staffs for kind consideration and excellent assistances.

REFERENCES

Berdalet, E., Fleming, L.E., Gowen, R., Davidson, K., Hess, P., Backer,L.C., Moore, S.K., Hoagland, P. and Enevoldsen, H. 2015. Marine harmful algal blooms, human health, and wellbeing: Challenges and opportunities in the 21st century. Journal of the Marine Biological Association of the United Kingdom 1: 1-31.

Blanco, J., Moroño, Á., Arévalo, F., Correa, J., Salgado, C., Pazos, Y., Calvo, S. and Rossignoli, A.E. 2022. Twenty-Five Years of PSP Toxicity in Galician (NW Spain) Bivalves: Spatial, Temporal, and Interspecific Variations. Toxins 14,837: 1-22.

Botelho, M.J., Vale, C. and Ferreira, J.G., 2019. Seasonal and multi-annual trends of bivalve toxicity by PSTs in Portuguese marine waters. Science of The Total Environment 664: 1095–1106.

Dechraoui Bottein, M.-Y. and Clausing, R.J. 2017. Receptor-binding assay for the analysis of marine toxins: Detection and mode of action. Comprehensive Analytical Chemistry 78: 277–295.

Etheridge, S.M. 2010. Paralytic shellfish poisoning: Seafood safety and human health perspectives. Toxicon 56: 108–122.

International Atomic Energy Agency. 2013. Detection of harmful algal toxins using the radioligand receptor binding assay. A Manual of Methods IAEA-TECDOC-1729, Vienna, Austria.

Kacem,I., Papiol G.G., de la Iglesia, P.,, Diogène, J., Hajjem, B. and Bouaïcha, N. 2015. Comparative toxicity and paralytic shellfish poisoning toxin profiles in the mussel *Mytilus galloprovincialis* and the oyster *Crassostrea gigas* collected from a Mediterranean lagoon in Tunisia: A Food Safety Concern. International Journal of Food Properties 18(5): 1075-1085.

Mafra, L.L. Jr., Bricelj, V.M. and Fennel, K. 2010. Domoic acid uptake and elimination kinetics in oysters and mussels in relationto body size and anatomical distribution of toxin. Aquatic Toxicology 100: 17–29.

Powell, C.L. and Doucette, G.L. 1999. A receptor binding assay for paralytic shellfish poisoning toxins: Recent advances and applications. Natural Toxins 7: 393-400.

Ruberu, S.R., Langlois, G.W., Masuda, M. and Perera, S.K. 2012. Evaluation of variability and quality control procedures for a receptor-binding assay for paralytic shellfish poisoning toxins. Food Additives & Contaminants: Part A 29: 1770–1779.

Ruberu, S.R., Langlois, G.W., Masuda, M., Kittredge, C., Perera, S.K. and Kudela, R.M. 2018. Receptor binding assay for the detection of paralytic shellfish poisoning toxins: Comparison to the mouse bioassay and applicability under regulatory use. Food Additives & Contaminants: Part A 35, 144–158.

Van Dolah, F.M., Finley, E.L., Haynes, B.L., Doucette, G.J., Moeller, P.D. and Ramsdell, J.S. 1994. Development of rapid and sensitive high throughput pharmacologic assays for marine phycotoxins. Natural Toxins 2: 189–196.

Van Dolah, F.M. 2000. Marine algal toxins: Origins, health effects, and their increased occurrence. Environmental Health Perspectives 108: 133–141.

Van Dolah, F.M., Fire, S.E., Leighfield, T., Mikulski, C.M. and Doucette, G.J. 2012. Determination of paralytic shellfish toxins in shellfish by receptor binding assay: Collaborative study. Journal of AOAC International 95, 795–812.

World Health Organization (WHO). 2010. International Food Safety Authorities Network (INFOSAN) Information Note No. 3/2010- Safety of street-vended food: Basic steps to improve safety of street-vended food.