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Full Length Research Paper

Simultaneous occurrence of saxitoxins and biogenic amines in mackerel fish

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Seafood toxins resulted from a wide variety of naturally occurring or man-made toxic compound may cause a serious hazard to consumers. Biogenic amines that cause histamine poisoning and (STX) that cause paralytic shellfish poisoning (PSP) are among these seafood toxins that may occur in mackerel fish and their presence may be affected by seasonal and locality variation. The presence of STX and biogenic amines together will increase the hazard to consumers. That is why the present study was conducted to explore the simultaneous occurrence of these toxins in mackerel fish in Egyptian markets. Also, to study the effect of catching areas and seasonal variation on the level of these toxins. Mouse bioassay method was used for STX determination, where the toxicity detected in mackerel fish in this study ranged from 21.4 to 45 µg STX/100 g. The quantitative Thin-layer chromatography (TLC) technique was applied for biogenic amines determination indicating that catching source had the main significant impact on the level of biogenic amines in mackerel fish samples under study. However, catching areas combined with seasonal variation affect the incidence level of STX in mackerel fish. Significant increase in the level of histamine (71), putrescine (64), cadaverine (42%), spermidine (65%), spermine (88%) and tyramine (48) was detected in the samples due to the thawing process.

Key words: Mackerel, biogenic amines, saxitoxins, thin-layer chromatography (TLC), paralytic shellfish poisoning (PSP).

INTRODUCTION

Marine food products are generally wholesome, nutritious, healthful and desirable. However, they may on occasion contain potent toxins of natural origin that could pose a significant threat to the health of consumers (Sindermann, 1996). Toxins formed by microorganisms may accumulate within certain tissues of predacious sea animals, which may serve as a source of seafood poisoning for the higher food chain. Such toxins are distinct from inorganic chemicals or infectious agents which may have contaminated the seafood (Watters, 1995; Van Egmond, 2004). Different chemical compounds resulted from different microorganisms are composing the array of the seafood toxins. Among these compounds are the saxitoxins (STX).

STX is a group of 16 compounds that produced mainly by species of *Gonyaulax* sp*., Alexandrium* sp*.* and other species of microorganisms (Kao, 1993; Van Egmond, 2004). Saxitoxins are selective sodium channel blocker in the nervous system resulting in rapid, paralysis and death. A dose of 0.2 mg is fatal to an average-weight human being (Shimizu et al., 1989; Hall et al., 1990). STX causes paralytic shellfish poisoning (PSP), a potentially fatal malady associated with the consumption of shellfish contained STX (Sephton et al., 2007). PSP is caused by the consumption of toxic shellfish (Shumway, 1990) and rarely by fin fish that have become toxic after feeding on STX-producing microalgae (Maclean, 1979).

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Abbreviations: PSP, Paralytic shellfish poisoning; **TLC,** thin-layer chromatography; **STX,** saxitoxins; **MU,** mouse units; **i.p.,** intraperitoneally.

Although, finfish unlike shellfish, die before the toxins reach dangerous levels in their flesh, however, some toxins (for example, STX), accumulate in the liver and other organs of some fish species. Mackerel is one of this species that can accumulate toxins and cause the death of other marine animal like what happen in 1987 where 14 humpback whales died suddenly in Cape Cod Bay (Massachusetts) after eating mackerel whose organs contained high concentrations of STX (Mons et al., 1998).

Biogenic amines can be formed in a wide variety of foods as results of amino acid decarboxylation. Histamine represents the major and the main cause of scombroid (histamine) poisoning, and other biogenic amine, such as tyramine, cadaverine, putrescine, tryptamine, and phenylethylamine acts as potentiates of histamine toxicity (Taylor, 1988; Al Bulushi et al., 2009; Joshi and Bhoir, 2011). Histamine, cadaverine, and putrescine have been found to be significant in fish safety and quality determination (Sultan, 2004; Al Bulushi et al*.,* 2009). Quality loss and histamine accumulation often occur after frozen fish of the mahi-mahi, bluefish, herring, and sardine are thawed and kept for long periods of time at room temperature before further processing. Since histamine is heat resistant, it can remain intact in canned or other processed fish products (Lopez-Sabater et al., 1994; Hungerford, 2010). An incident of food borne poisoning causing illness in three victims due to ingestion of canned mackerel that contained histamine greater than the USFDA allowable limit of 5 mg/100 g occurred in December 2001, in Taipei Prefecture, northern Taiwan (Tsai et al*.*, 2005; Lokuruka, 2009).

Toxicity associated with the consumption of the scombroid fish is called scombrotoxicosis which include different toxic compounds. Synergistic effect of these compounds has been reported by Clifford et al. (1993) who found that the toxicity in mackerel of the involved toxic compounds is higher than the reported toxicity of each compound separately.

Although, the presence of STX and biogenic amines in mackerel fish has been recorded in several reports, and although, in the Egyptian market where mackerel fish considered being one of the most popular fish and where this type of fish is mainly imported from different catching areas in Europe and Asia along with smaller amount brought locally from the Egyptian Mediterranean costal area, however, no work has been done before to explore the safety of this fish in the Egyptian market. Therefore, the main objective of the present study was to explore the simultaneous occurrence of these toxins in mackerel fish in Egyptian markets. Also to study the effect of catching areas and seasonal variation on the level of these toxins.

MATERIALS AND METHODS

Sampling

A total of 60 mackerel fish samples each sample consists of 3 fishes (a total of 180 fish) were collected from the Egyptian market to represent 5 catching areas (Holland, Japan, China, Korea and Egypt) during winter, spring, summer and autumn. All the information reported in this study about the catching source and time was retained from the package label. However, the word season reported in this study is referred to the collection time rather the catching time reported in the package label. Although in case of STX the catching time is more important than the marketing time as this toxin is more related with the algal bloom in the catching area and has nothing to do with the handling process after catching, however, the level of biogenic amines is more related with all the process after catching. This is why in this study the collection time is used rather than the catching time and the information about the catching time was used in the discussion of the results.

The flesh of each sample was divided into 3 portions the first to determine the biogenic amines content in fish samples and the second to study the effect of thawing process on the level of biogenic amines. The third flesh portion along with the liver of the mackerel samples was used to determine STX.

Saxitoxin determination

Mackerel's liver and flesh of collected samples were weighed and 100 g of each were extracted by 0.1 N HCl and blended for 2 min. The mixture was boiled for 5 min, kept at room temperature to cool down and pH was adjusted to 3.0 using 5 N HCl. The mixture was then centrifuged at 4500 g for 10 min and the supernatant was retained for mouse assay toxicity test (AOAC, 2007). Albino Swiss male mice with average weight 18 to 22 g were used for toxicity assessment. Mice were obtained from Animal Housing Division in the National Research Center, Cairo, Egypt. Potency was expressed as Mouse units (MU). The survival time was measured from the completion of the intraperitoneally (i.p.) injection till the last breath of the mice (AOAC, 2007).

Determination of biogenic amines level

Histamine, putrescine, cadaverine, spermidine, spermine and tyramine, were extracted and determined in all tested samples according to Maijala and Eerola (1993). The dansylated derivatives of the amines were formed by dissolving the residue with 0.5 ml of saturated $NaHCO_3$ solution, then adding 1 ml dansyl chloride solution (500 mg/100 ml acetone).

TLC technique was used to separate the six dansylamines under investigation (Figure 1). The marked dansylamines areas were determined using SHIMADZU CS-9000 Dual wavelength flying spot scanning densitometer at 254 nm wavelength. Standard curve of each dansylamine was used to calculate the concentrations of biogenic amines in the tested samples.

Statistical analysis

The data were subjected to the proper statistical analysis using Mstat-C program (Mstat, 1988). For means comparison Duncan's multiple range test was applied at 5% level.

RESULTS AND DISCUSSIONS

Toxicity determination of saxitoxins

Mouse bioassay is used in this study to determine STX and STX-like compounds because it is an official method

Table 1. Mouse unites and saxitoxin equivalent after i.p. injection by extraction of mackerel liver samples collected from different sources in different seasons.

Catching source	Toxin concentration	Winter	Spring	Summer	Autumn
Japan	MU/100 g Mean ± SE	---	---	112 ± 2.3	---
	µg STX /100 g	---	---	22.4	---
China	MU/100 g Mean ± SE	---	207 ± 3.8	136 ± 3.7	---
	µg STX /100 g	---	41.4	27.2	---
Korea	MU/100 g Mean ± SE	---	107 ± 1.8	124 ± 3.4	---
	µg STX /100 g	---	21.4	24.8	---
Holland	MU/100 g Mean ± SE	225 ± 15	---	---	---
	µg STX /100 g	45	---	---	---
Egypt	MU/100 g Mean ± SE	---	144 ± 2.9	---	---
	µg STX /100 g	---	28.8	---	---

and is capable to detect a wide variety of seafood toxins regardless its chemical structure. However, this method cannot detect biogenic amines.

Although not all samples cause death to mice, however, all flesh and liver samples in all seasons and from all localities showed neurosigns after i.p. injection in mice which means that all samples contain concentration of STX and STX-like compounds but in flesh samples and some of the liver samples this concentration was not high enough to cause death to mice.

Only 35% of the liver samples showed detectable amount of STX (Table 1). Samples imported from Holland in winter season were the only samples that cause death to mice in a mean death time of 4:15 min which equivalent to 225 MU/100 g wet weight and which is equivalent to 45 µg STX eq. /100g wet weight (Table 1). Local samples from Egypt showed detectable amount of STX (mean death time of 6:27 min which is equivalent to 144 MU /100g or 28.8 µg STX) only in spring season. Whereas, samples imported from Asia showed detectable toxicity in spring (China 41.4 µg STX and Korea 21.4 µg STX) and summer (Japan 22.4 µg STX, China 27.2 µg STX, and Korea 24.8 µg STX). All samples showed non-detectable amount of STX in autumn.

The toxicity detected in mackerel fish in this study with a concentration ranged from 21.4 to 45 µg STX confirmed several reports of the accumulation of STX in mackerel fish where STX concentration ranged from 40 to 209 µg STX were found in Atlantic mackerel (*Scomber scombrus*) of the Bay of Fundy, New Brunswick, Canada in summer of 1988 (Haya et al., 1990) and in the Gulf of St. Lawrence (Castonguay et al*.,* 1997). Also STX of an unknown origin was reported by Shimizu et al. (1989) in the common mackerels, *S. scombrus* Linn.

This results that showed neurosigns in mice of all flesh and liver samples confirmed the fact that mackerel fish accumulates STX mainly in the liver and in non-detectable amount in flesh all year round was also reported by Castonguay et al. (1997) who stated that mackerel appears to retain toxins (saxitoxins, gonyautoxins) year-round. However, we could not confirm their finding that the toxin content of liver increased significantly with fish age and length suggesting that mackerel progressively accumulate PSP toxins throughout their life. Also their finding that the toxin content of the liver also increased significantly during the summer feeding was disagreed with our finding that different localities showed different pattern of toxin accumulation of STX in mackerel which reflect the pattern of the toxic algal bloom in these localities. The toxic winter samples from Holland may suggest the occurrence of late fall toxic bloom in the fishing area where these samples were caught. Also the spring and summer toxin accumu-lated in mackerel samples from Asia and the spring toxin accumulation in local samples from the Mediterranean showed different pattern of toxic bloom in different geographical areas. This information may be useful to those who monitor the safety of imported food to thoroughly monitored mackerel fish from certain localities in certain time.

Although all positive samples showed toxin concentrations ranged from 21.4 to 45 µg STX eq. /100g wet weight which is below the safe limit of 80 µg STX eq. /100 g wet weight set by the USFDA and the EU Directive action 91/492/EEC, however, this may not be always the case and the possibility of getting higher toxic bloom and consequently higher toxin accumulation in mackerel is always there. Also the fact that accumulation of toxin is mainly in liver and rarely in flesh is not safe enough for consumption with the possible contamination with the toxin in liver during certain preparation and cooking procedures.

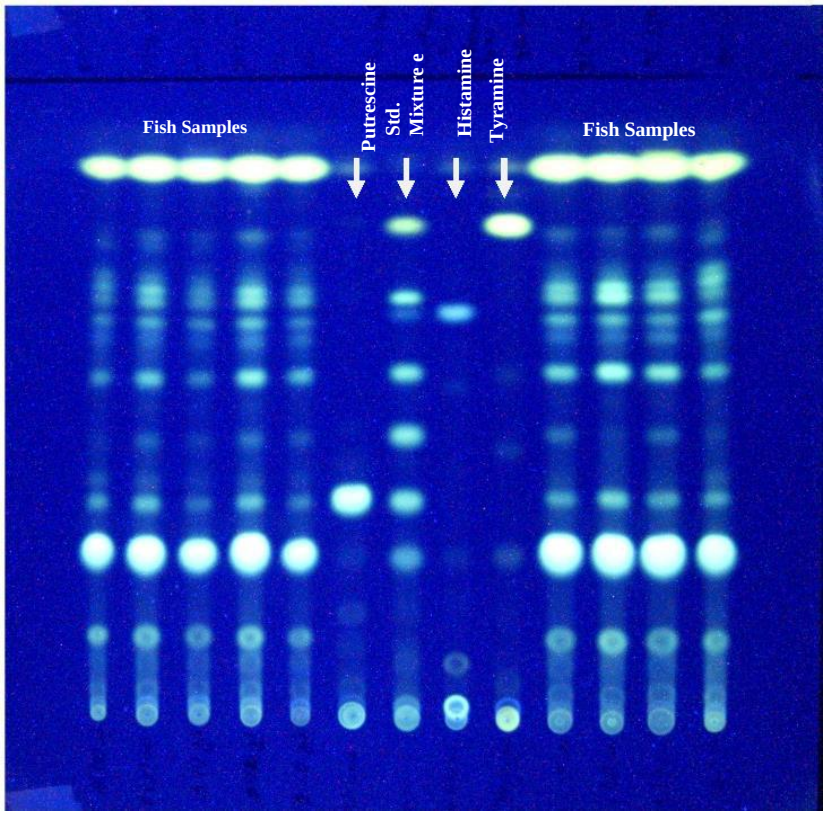

Figure 1. TLC separation of the tested biogenic amines (putrescine, cadaverine, spermidine, histamine, spermine and tyramine) from mackerel fish samples separated spots were visualized using long wave UV (365 nm).

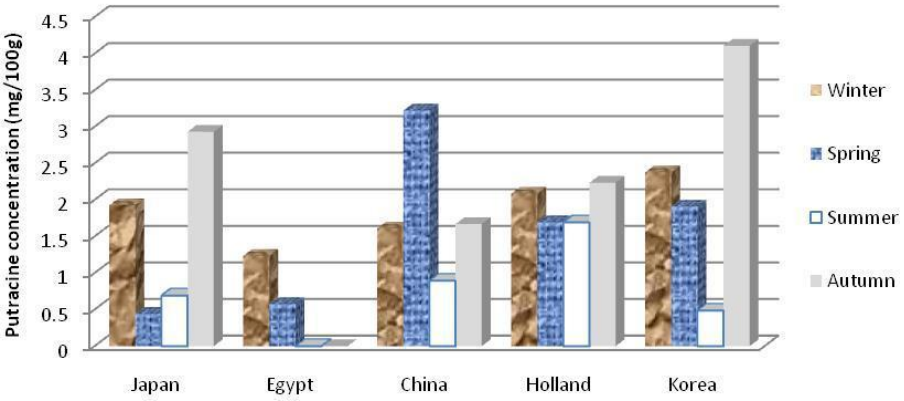

Figure 2. Effect of catching source on the content of putrescine in mackerel fish samples collected from the Egyptian markets during different seasons.

Biogenic amines determination

Effect of seasons

Statistical analysis of the data in this study showed that there are significant differences between seasons concerning the level of biogenic amines in mackerel fish samples. Winter season showed the significant highest mean level of histamine (7.198), spermidine (5.18) and cadaverine (3.035) mg/100g. In spring season, spermine (3.718) and tyramine (2.219) mg/100 g were at their highest level whereas putrescine (2.189 mg /100 g were at its highest level in autumn (Figures 2, 3, 4, 5, 6 and 7). Unexpectedly the lowest level of biogenic amines under

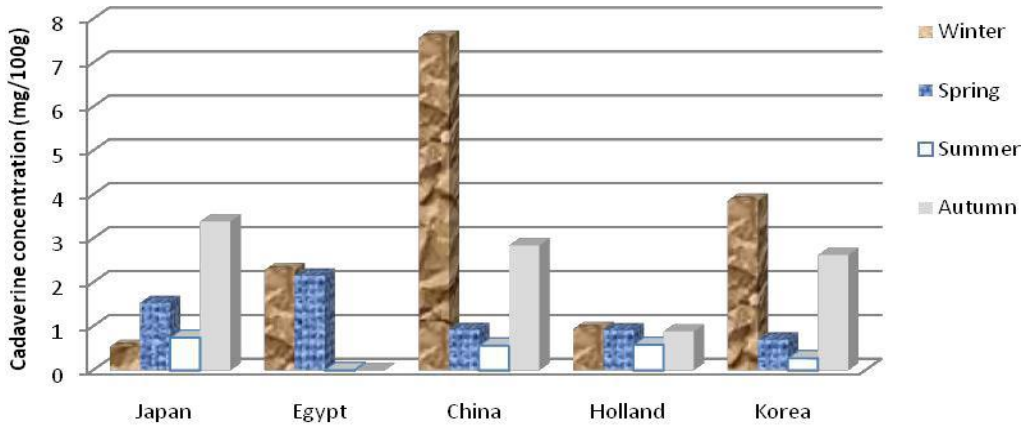

Figure 3. Effect of catching source on the content of cadaverine in mackerel fish samples collected from the Egyptian markets during different seasons.

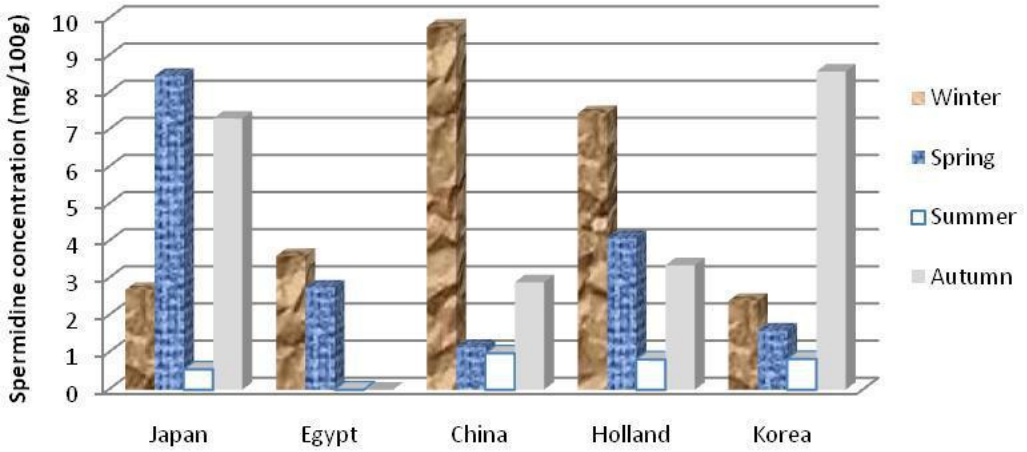

Figure 4. Effect of catching source on the content of spermidine in mackerel fish samples collected from the Egyptian markets during different seasons.

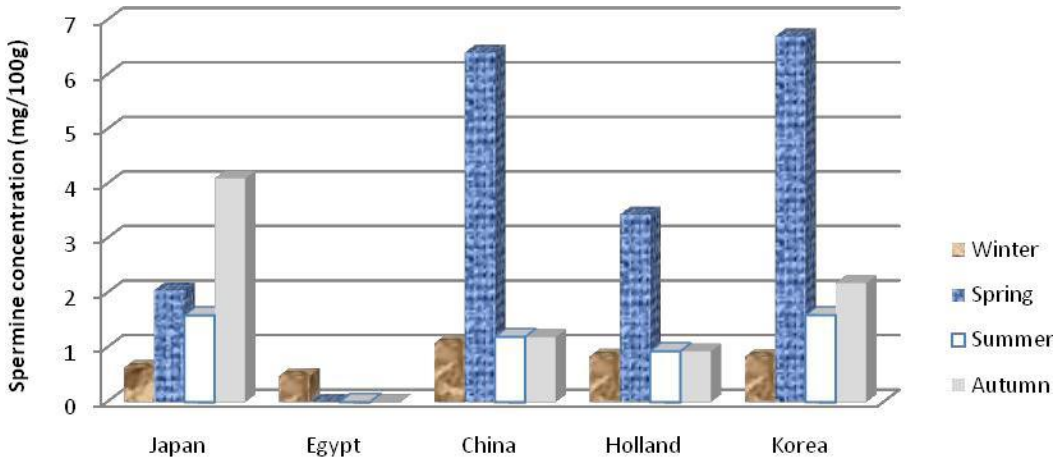

Figure 5. Effect of catching source on the content of spermine in mackerel fish samples collected from the Egyptian markets during different seasons.

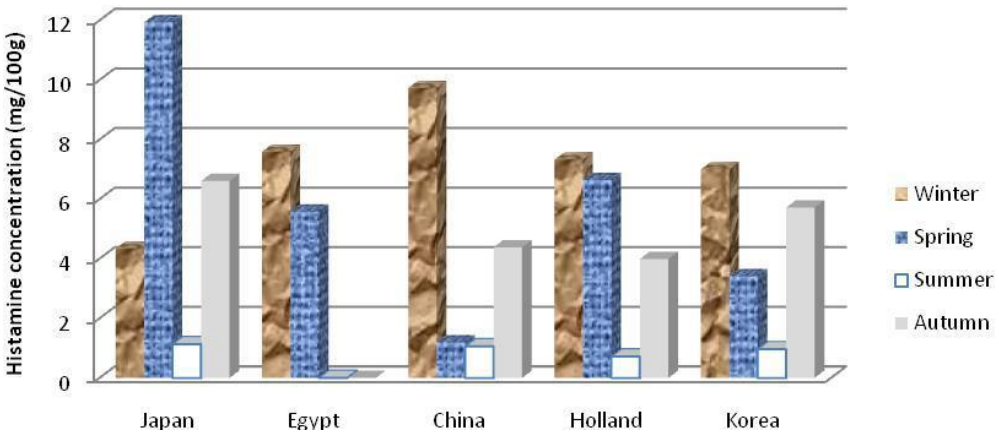

Figure 6. Effect of catching source on the content of histamine in mackerel fish samples collected from the Egyptian markets during different seasons.

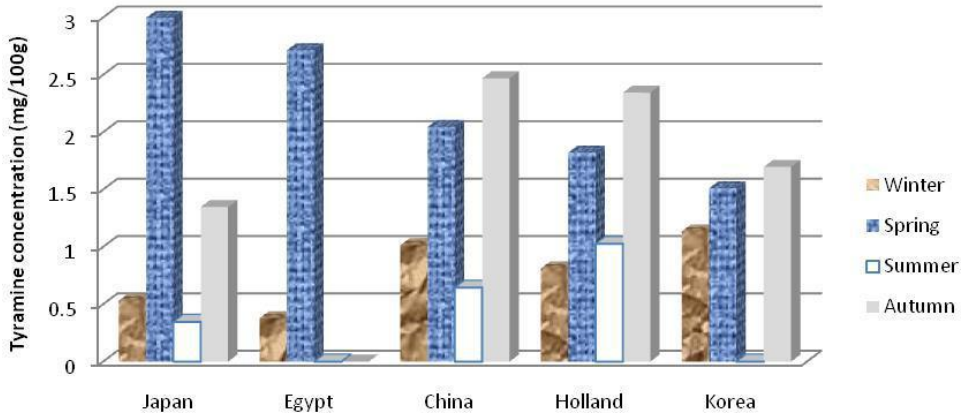

Figure 7. Effect of catching source on the content of tyramine in mackerel fish samples collected from the Egyptian markets during different seasons.

study was detected in summer samples. This finding is in contrast with several reports showing that biogenic amine levels in summer were greater than those in winter and that increasing holding temperature during handling will increase the biogenic amines content (Klausen and Huss, 1987; Ben-Gigirey, 1998; Yiyeh et al., 2004; Park et al., 2010). This disagreement may due to the common practice in the local market where frozen fish are handled with high care during summer time, whereas lower temperature around 20°C during winter time makes the market deal with frozen fishes with less fear of getting their merchandise to be spoiled.

Effect of catching source

With regard to the effect of catching source on the level of biogenic amines, the present data showed that mackerel fish were significantly differ in their biogenic amines level due to their catching source. The obtained results indicated that mackerel fish from Asia catching areas contained significant higher level than other catching areas. The highest mean level of histamine and spermidine (4.747) mg/100g were detected in samples imported from Japan, while cadaverine (2.972) mg% and tyramine (1.544) mg% were recorded in China samples, whereas, samples from Korea showed the highest level of putrescine (2.22) and spermine (2.825) mg% (Figures 2, 3, 4, 5, 6 and 7). The effect of catching source is mainly due to the type and level of microorganisms which affect histamine level (Rawles et al., 1996) and is also due to harvesting season and feeding activity prior to capture as they affect the biogenic amines precursor (Aksnes and Brekken, 1988).

The effect of thawing process

Biogenic amines are normally increased rapidly during uncontrolled thawing process of frozen mackerel. The

Table 2. Effect of thawing process on the level of biogenic amines (mg/100 g) in mackerel fish samples.

Effect	Putrescine	Cadaverine	Spermidine	Spermine	Histamine	Tyramine
Frozen	201.1 ±.15^{b}	20.13±0.22^{b}	106.2±0.41^{b}	1.255±0.18^{b}	3.302±0.41^{b}	1.003±0.13^{b}
Thawing	20216±0.22^{a}	20211±0.29^{a}	4.311±0.56^{ab}	2.357±0.39^{a}	5.632±0.65^{a}	1.482±0.16^{a}
% Increase	64	42	65	88	71	48

Means in the same column bearing different letters differ significantly at p>0.05 otherwise they differ non significantly from each other.

Table 3. Effect of interaction of thawing process and season on the level of biogenic amines content (mg/100 g) in mackerel fish samples.

Interaction	Putrescine	Cadaverine	Spermidine	Spermine	Histamine	Tyramine
Winter*F	1.481±0.42^{c}	2.711±0.65^{ab}	4.144±0.88^{b}	0.473±0.07^{d}	5.765±0.95^{b}	0.605±0.11^{de}
Winter*T	1.655±0.33^{bc}	3.359±0.85^{a}	6.215±1.28^{a}	1.066±0.10^{d}	8.632±1.25^{a}	0.945±0.12^{cd}
Spring*F	1.370±0.18^{c}	1.037±0.27^{cd}	2.115±0.53^{cd}	2.345±0.47^{b}	3.573±0.74^{c}	1.914±0.25^{b}
Spring*T	2.316±0.26^{ab}	1.448 ±0.27^{c}	5.101±1.14^{ab}	5.090±1.21^{a}	7.937±1.47^{a}	2.524±0.24^{a}
Summer*F	0.477±0.11^{d}	0.326±0.07^{d}	0.395±0.09^{d}	0.970±0.24^{d}	0.669±0.17^{d}	0.255±0.08^{e}
Summer*T	1.029±0.26^{cd}	0.533 ±0.12^{d}	0.866±0.17^{d}	1.149±0.27^{d}	0.880±0.18^{d}	0.553±0.14^{de}
Autumn*F	1.474±0.35^{c}	1.416±0.33^{c}	3.783±1.05^{bc}	1.235±0.34^{cd}	3.203±0.67^{c}	1.239±0.26^{c}
Autumn*T	2.903±0.66^{a}	2.477± 0.52^{b}	5.062±1.06^{ab}	2.122±0.51^{bc}	5.077±0.84^{bc}	1.905±0.39^{b}

Means in the same column bearing different letters differ significantly at p>0.05 otherwise they differ non significantly from each other. F=Frozen T=Thawed.

result in this study confirmed this fact where the thawing process causes the significant increase in the level of the biogenic amines under study (Table 2). Although this increase in the level of biogenic amines was found to be significant in all tested amines, however, the percent increase was differed according to the type of biogenic amine (Table 2). The highest percent increase was found in spermine (88%); followed by histamine (71%) where the lowest percent increase was observed in cadaverine (42%). Variation in the formation of different amines at different temperatures was probably due to the effect of temperature on the growth and activity of the amine forming bacteria as well as the availability of the amino acid substrates (Yamanaka et al., 1986; Okuzumi et al., 1990; Chong et al., 2011).

Statistical analysis showed significant interactions between thawing process and both catching seasons and sources. Both interactions confirmed that the higher the initial contamination the higher the percent increase due to the thawing process. This finding was illustrated in Table 3, where the highest initial histamine found in winter season cause the highest significant percent increase (50%) due to the thawing process while the lowest initial histamine found in summer season was not enough to cause a significant percent increase (31%). The same trend was observed in the effect of the thawing process on the level of spermidine and cadaverine. Also, this was true in case of spermine and tyramine when comparing their levels in the spring and summer season and for putrescine in autumn compared to summer (Table 3).

The effect of high initial concentration on the percent increase during thawing process was not observed in the case of the interaction of the thawing process and catching source (Figures 8, 9, 10, 11, 12 and 13). The samples imported from Asia which showed the significant higher initial levels did not showed the higher percent increase (for example, Japan samples showed 73% increase in histamine and 66% in cadaverine), however the higher percent increase was observed in samples from Holland as indicated for histamine (145%) and cadaverine (157%) . This may due to other factors related to the catching source (for example, differences due to microflora species and/or the substrate type) as was discussed in previous reports where several studies showed that amino acid formation that depends on the feeding activity prior to capture and the high content of proteolytic enzymes in the intestinal tract are responsible for the rapid autolysis process (Gildberg, 1978; Aksnes, and Brekken, 1988).

Conclusions

Accordingly, in this study we were able to detect both STX and biogenic amines in the same samples of mackerel fish which increase the hazard of these toxic compounds if they occur separately as reported by Clifford et al. (1993) where they found that, commercial mackerel fillets which have been associated with

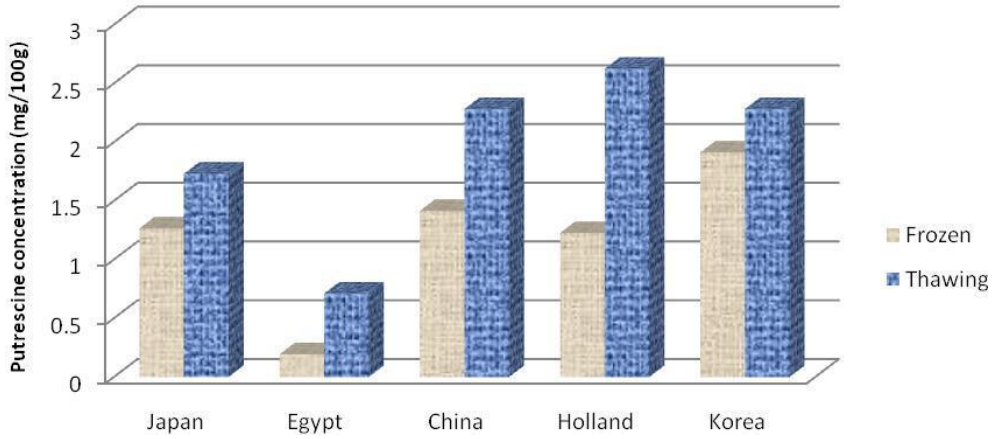

Figure 8. Effect of thawing process on the content of putrescine in mackerel fish samples collected from the Egyptian markets.

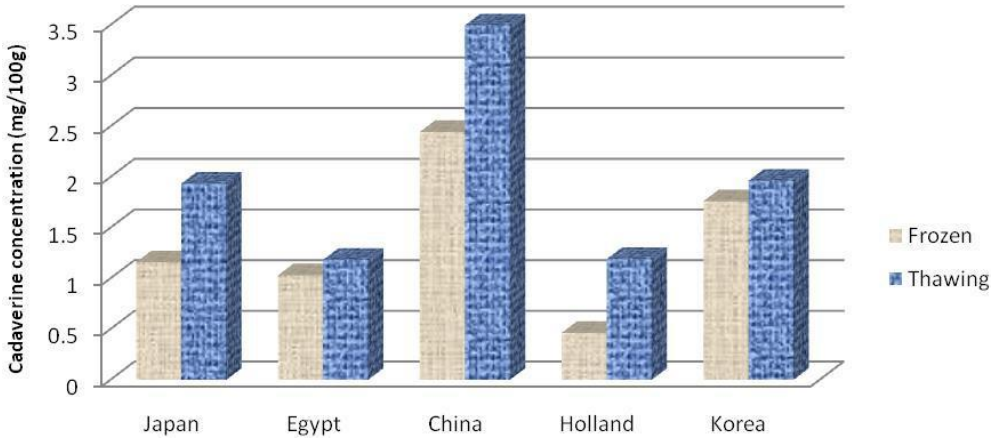

Figure 9. Effect of thawing process on the content of cadaverine in mackerel fish samples collected from the Egyptian markets.

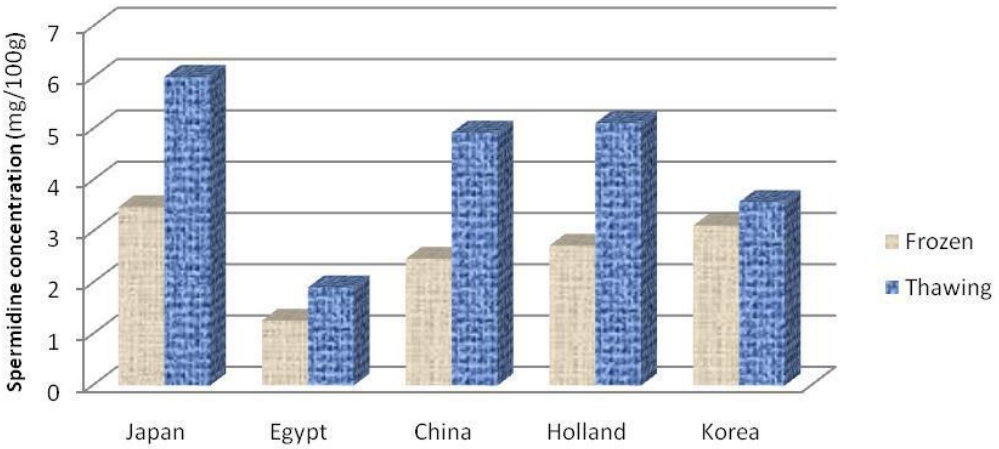

Figure 10. Effect of thawing process on the content of spermidine in mackerel fish samples collected from the Egyptian markets.

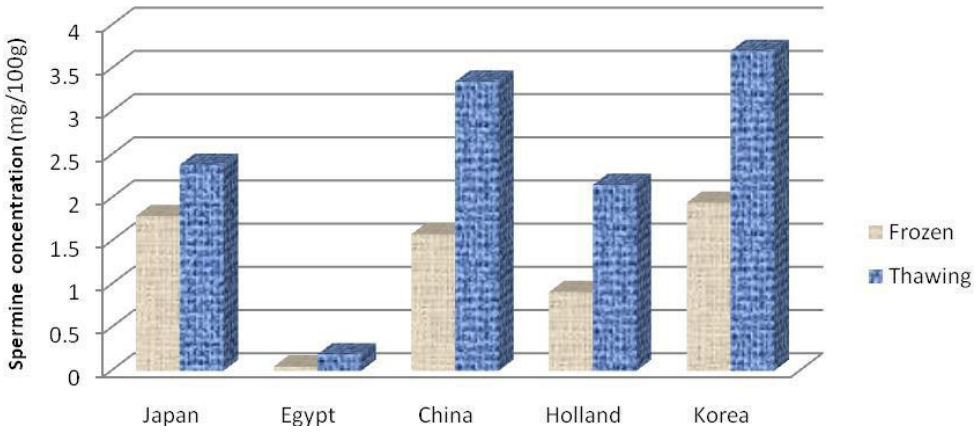

Figure 11. Effect of thawing process on the content of spermine in mackerel fish samples collected from the Egyptian markets.

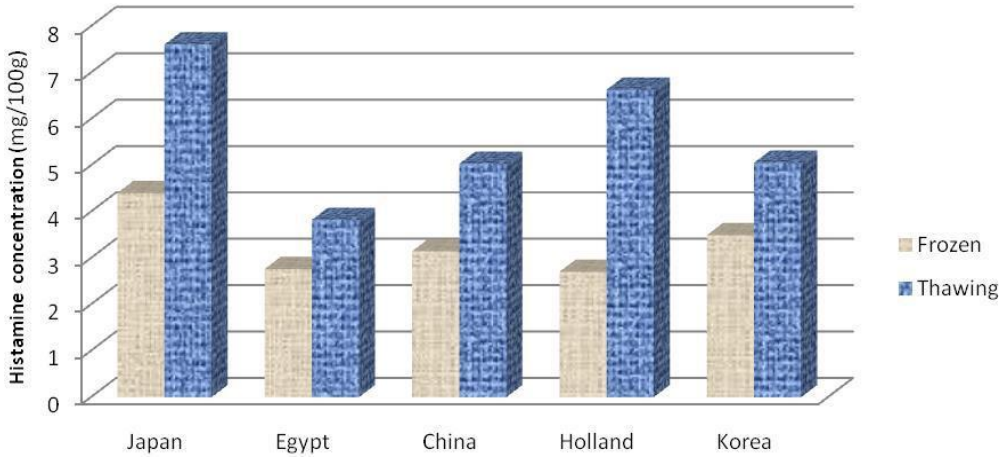

Figure 12. Effect of thawing process on the content of histamine in mackerel fish samples collected from the Egyptian markets.

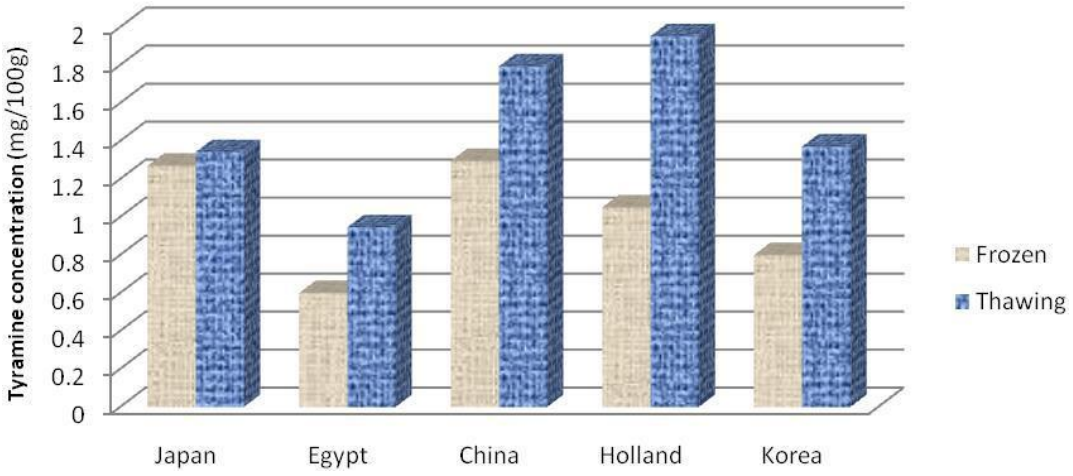

Figure 13. Effect of thawing process on the content of tyramine in mackerel fish samples collected from the Egyptian markets.

incidents of scombrotoxicosis contained 0.2 to 13 µg STX/ 100g, concentrations some two to four orders of magnitude below that normally detectable by the mouse bioassay, concluding that the synergistic action of different toxic compound may be responsible for this incidents of scombrotoxicosis.

In general mackerel fish in this study collected in autumn season contained low level of biogenic amines and non detectable level of STX while in summer STX was at its higher level in the samples from Asia but the biogenic amines was at its lowest level. In spring and winter time the hazard of the presence of these toxins simultaneously is more likely to occur as for the samples from Asia that contained high level of STX and high level of spermine and Tyramine (Table 1 and 3). Also the samples from Holland in winter time showed high level in STX and all the tested biogenic amines.

The objective of this study was mainly to detect the increasing hazard due to the presence of more than one toxic compound at the same time in mackerel fish with relation to the catching source and season. This objective was supported by the result obtained in this study where both STX and biogenic amines under study were detected in the same samples of mackerel fish. Therefore this data should set the alarm of reviewing the safe limit of different toxic compound that may occur simultaneously in a specific product.

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