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Microbial Status and Formation of Biogenic Amines in Salted Fish: A Study for Their Dietary Intake and Health Risk Assessment

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Abstract

Salted fish is a common food type in Egypt that is consumed in certain occasions and celebrations. The manufacture process of the salted fish allows fermentation of different fish species. Therefore, there is a high chance for microbial growth and multiplication on such rich protein substrate, and decomposition of the amino acids to their biogenic amines (BAs) derivatives. This study was undertaken to estimate the formed BAs in three salted fish retailed in Egypt, named feseikh, sahlia, and salted sardine. In addition, microbial counts including total plate counts (TPC), total psychrophilic counts (TPsC), and total Staphylococcus aureus counts (TSC) were enumerated. Dietary intakes and health risks associated with the formed BAs were also calculated and discussed. The obtained results revealed formation of the BAs in all examined salted fish species. Feseikh had the highest BAs contents as well as microbial counts. In conclusion, although the calculated dietary intakes revealed no potential risks associated with the consumption of the salted fish, however this assumption should be handled carefully due to inter-individual differences in their immune reactions to such BAs, particularly histamine.

KEYWORDS
Salted fish, Biogenic Amines, Health risks, Histamine

INTRODUCTION

Fish is regarded as a significant source of protein, low-cholesterol, high-polyunsaturated fatty acids, trace element, and vitamin. Globally, there is a growing need for fish consumption to make up for the scarcity of red meat (Morshdy *et al*., 2019; 2021). Fish is a highly perishable source of protein. When it is transported, handled, and processed under unhygienic conditions, microbiological contamination and fish deterioration may happen, which can result in the creation of a wide range of compounds (Huss, 1995; El-Ghareeb *et al*., 2021). Salted fish such as feseikh, sardine, and sahlia are highly popular fish products in Egypt and particularly consumed in special occasions and celebrations of the country (Morshdy *et al*., 2022).

Natural toxicants known as biogenic amines (BAs) are created by a wide range of bacteria, some of which are pathogenic and some of which are not, as well as by the metabolism of specific amino acids. According to Marcobal *et al*. (2012), BAs are divided into two categories: 1) monoamines, which include histamine (HIS) and tyramine (TYM), are created by a one-step decarboxylation reaction for their source amino acids, histidine and tyrosine, respectively. 2) Diamines, such as putrescine (PUT) and cadaverine (CAD), which are created when lysine undergoes a decarboxylation process. The latter is a diamine that can be made indirectly following arginine hydrolysis or directly through a single-step decarboxylation for ornithine and agmatine (Wunderlichová *et al*., 2014). 3) A number of processes, including the hydrolysis of arginine into ornithine and agmatine, result in the production of polyamines like spermine (SPM) and spermidine (SPD) (Shah and Swiatlo, 2008). At physiological quantities, biogenic amines are crucial for a number of cellular functions, such as gene expression, cell division, and tissue repair (Benkerroum, 2016; Galgano *et al*., 2012; Ma *et al*., 2020). It is unlikely that consuming large amounts of these BAs will cause serious side effects such allergy, hypertension, neurological manifestation, or even death (Medina *et al*., 2003). Furthermore, certain BAs, such PUT and CAD, have been linked to the development of gastric cancer because they can be transformed by digestive tract microbes into carcinogenic N-nitroso compounds (Koutsoumanis *et al*., 2010). According to the EFSA Panel on Biological Hazards (BIOHAZ) report (EFSA, 2011), eating fish that has high amounts of BAs has been linked to intoxication incidents in Europe.

Salted fish quality and hygiene are thought to be well-indicated by screening for the presence of BAs in such fish products (Koutsoumanis *et al*., 2010; Benkerroum, 2016). The amounts of BAs in the various types of fish that are sold in Egypt and how much of them go toward the population's estimated daily intake of BAs are conspicuously lacking in information. Moreover, there is less information in the correlation study between the microbial numbers and the production of BAs. Thus, the primary goal of the current study was to estimate the produced BAs in three major salted fish types retailed in Egypt, namely feseikh, sardine and sahlia. The population of Egypt's estimated daily intakes of BAs resulting from the ingestion of these types of fish were also com-

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puted. Additionally, the fish samples were subjected to microbial counts, such as total plate counts (TPC), total staphylococcus aureus (TSC) and total psychrophilic counts (TPsC), and their associations with total biogenic amines (TBAs) were examined.

MATERIALS AND METHODS

Collection of fish samples

Ninety salted fish samples (*n.*= 30 of each species) were randomly selected from local markets in Egypt. The salted fish samples included feseikh, salted sardine, and sahlia. The gathered samples were sent straight to the laboratory of Food Hygiene, Animal Health Research Institute, Mansoura Branch after being chilled, where they were examined microbiologically and for their BA contents.

Biogenic amine content

Each sample was first homogenized using 10 grams and 100 milliliters of 10% trichloroacetic acid. The homogenates were then extracted for an hour, and after that, they were centrifuged for 20 minutes at 5,000 rpm and 4ºC. Whatman filter No. 1 was used to filter the supernatants, and the filtrates were stored at 4ºC until analysis. According to Kononiuk and Karwowska (2019), an amino acid analyzer (L-8900, HITACHI, Japan) was employed for the quantitative estimation of BAs. The amine standards (Merck KGaA, Darmstadt, Germany) were used to determine the contents of the biogenic amines, including HIS, TYR, PUT, CAD, SPM, and SPD. The concentrations of biogenic amines were expressed as mg/kg of salted fish.

Daily BA intake

The following formula was used to determine Egypt's estimated human daily intake (EDI) of the total biogenic amines from salted fish consumption: EDI = Ci x FIR.

Where IR is the fish ingestion rate in Egypt and Ci is the concentration of either individual or total BAs in the fish. According to FAO (2003), Egypt's fish ingestion rate (FIR) is 48.57 g/day.

Total microbial counts

Using the APHA (2001) guidelines, the total plate (TPC), total psychrophilic (TPsC) and total Staphylococcus aureus (TSC) counts, were performed. To put it briefly, 225 milliliters of sterile buffered peptone water 0.1% was homogenized with 25 grams of each salted fish sample, spinning at 2500 rpm for two minutes. These homogenates show the 10-1 dilution, after which decimal dilutions were made. One milliliter (ml) of each dilution was put into each of two sterile Petri dishes, along with fifteen milliliters of nutrient agar (Oxoid) for TPC, and TPsC, or Baired Parker media for TSC. The inoculation plates were thoroughly mixed and allowed to solidify before being incubated for 24 hours at 37°C for TPC, and TSC or 10 days at 7ºC for TPsC. Following calculations and recording, the agar plates with 30–300 pinpoint colonies were identified:

TPC, TSC, or TPsC/g = average number of colonies × dilution reciprocal Log_{10} cfu/g is the stated colony count.

Statistical analysis

All data were reported as means±SE and measurements were performed in duplicate. To assess the statistical analysis, the Tukey-Kramer HSD test was used. SAS Institute Inc., Cary, NC's JMP statistical package was used to do a Pearson correlation study. Using the JMP statistical program from SAS Institute Inc., Cary, NC, statistical significance was determined in all analyses with a $P<0.05$.

RESULTS AND DISCUSSION

Six BAs were measured in three different types of salted fish that are sold in Egypt for the current investigation. The type of fish and their microbiological state had a major impact on the created individual and total BAs.

Histamine

The findings showed that HIS production occurred in every fish sample that was analyzed. HIS concentrations in all fish species were below the 100 mg/kg maximum permitted limit (MPL) (FDA 2011). Feseikh had the greatest mean concentrations of HIS (77.2±0.39 mg/Kg) with a significant difference ($P<0.05$), followed by sahlia (22.55±0.74 mg/Kg) and salted sardine (48.55±1.58 mg/Kg) (Fig. 1A). The HIS concentrations found in the current study were similar to those found in sardine and mackerel sold in Egypt (Sabry *et al*., 2019) and Peru (Gonzaga *et al*., 2009) as well in Korea (Kang *et al*., 2019). Sardine from Serbia was shown to have higher HIS levels (>300 mg/Kg) (Petrovic *et al*., 2016). Conversely, in a Polish study, Tilapia had decreased HIS levels (Kulawik *et al*., 2013). In Egypt, the EDI values of HIS from eating salted fish species varied from 1.095 mg/day for sahlia to 3.459 mg/day for feseikh (Table 1). Higher levels of HIS in fish can cause scombroid poisoning, also known as HIS-intoxication, which manifests as hot flushes, chest pain, nervous system symptoms, cardiovascular symptoms, gastrointestinal issues, and anaphylaxis (FAO/WHO 2012). The no known adverse effect threshold (NOAEL) of HIS was set at 50 mg by the EFSA panel on biological hazards (EFSA, 2011). This could suggest that there will be no negative consequences on the human population from the HIS measured EDI levels in the current investigation. However, eating raw or processed fish has been linked to multiple HIS-intoxication epidemics documented in Taiwan (Chen *et al*., 2010). Similar to this, episodes of HIS-intoxication were documented in Australia from 2001 to 2013, primarily as a result of tuna eating (Knope *et al*., 2014). Additionally, in a kindergarten located in the Vojvodina province of northern Serbia, an outbreak of HIS-intoxication was documented involving children who had consumed canned sardines (Petrovic *et al*., 2016). This illustrates the inter-individual variability in their vulnerability to the onset of HIS poisoning symptoms. Variations in age, dietary habits, and genetic predisposition could potentially account for this discrepancy (Visciano *et al*., 2014).

Table 1. Estimated daily intakes (mg/day) of biogenic amines via consumption of salted fish in Egypt.

	Sahlia	Feseikh	Salted sardine
Cadaverine	0.1	0.40	0.28
Histamine	1.10	3.46	2.36
Putrescine	0.08	0.34	0.26
Spermine	0.07	0.27	0.16
Spermidine	0.19	0.25	0.24
Tyramine	1.69	3.65	2.92
Total BAs	3.23	8.37	6.22

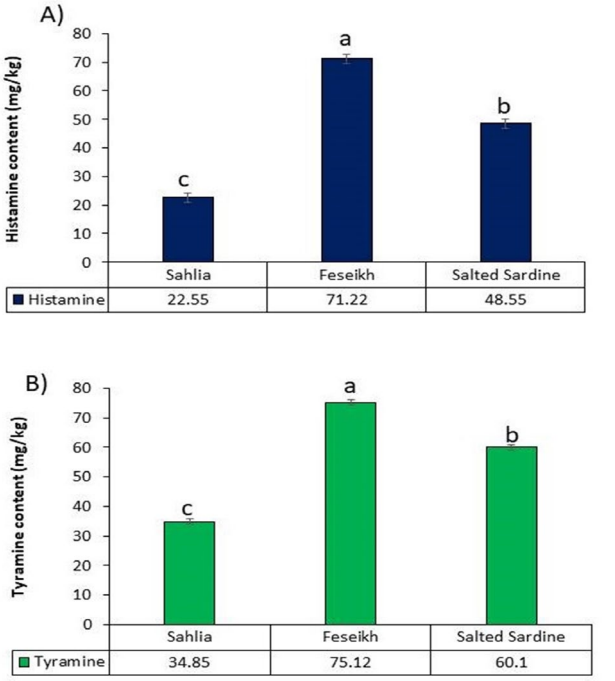

Fig. 1. A) Histamine, B) Tyramine contents (mg/kg) in the examined sahlia, feseikh, and salted sardine. Columns with different letter are significantly different at ($p< 0.05$).

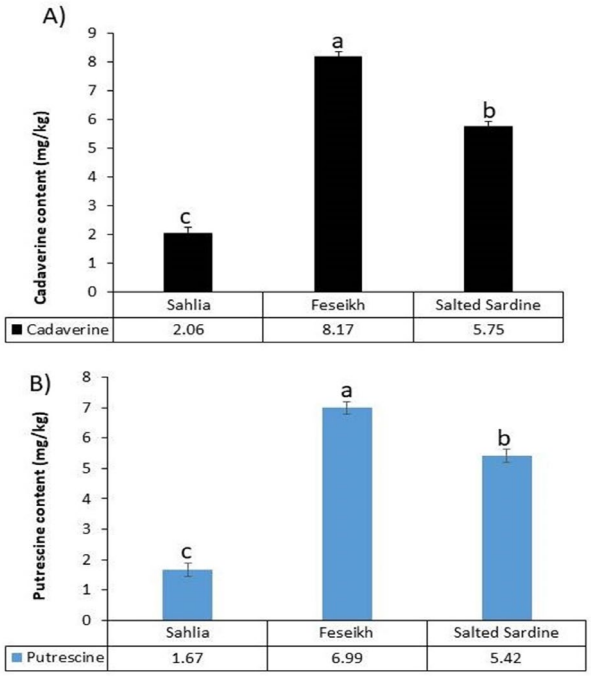

Fig. 2. A) Cadaverine, B) Putrescine contents (mg/kg) in the examined sahlia, feseikh, and salted sardine. Columns with different letter are significantly different at ($p< 0.05$).

Tyramine

All of the salted fish samples that were analyzed for this investigation have TYR, however, at levels that is below the suggested MPL (100 mg/kg) (Silla Santos, 1996). The largest residues of TYR (75.12±0.88 mg/Kg) were found in Feseikh, followed by sahlia (34.85±0.55 mg/Kg) and salted sardine (60.10±0.85 mg/Kg) (Fig. 1B). Comparable amounts of TYR were found in tinned Mackerel (Weremfo *et al*., 2020), Mackerel (Kim *et al*., 2009), Sardine (Özogul and Ṛzogul 2006), Herrings (Özogul *et al*., 2002), and Mackerel (Kim *et al*., 2009). According to Table 1, the estimated EDI values for TYR varied from 1.692 mg/day in sahlia to 3.648 mg/day in feseikh. A maximum acceptable level of TYR in Austria for high fish diet was proposed by Paulsen *et al*. (2012) at 950 mg/Kg. This implies that the people of Egypt would not be negatively impacted by the levels found in the current study. That being said, TYR is a recognized vasoactive biogenic amine that has been connected to cardiac failure, elevated heart rate, intracranial bleeding, and hypertension crises (Benkerroum, 2016). Thus, extreme caution is required, especially for those who are extremely vulnerable.

Cadaverine

In every salted fish sample that was analyzed, CAD was found at quantities lower than the MPL (540 mg/kg) (EFSA, 2011). According to Figure 2A, feseikh had considerably ($P<0.05$) higher CAD levels (8.17±0.07 mg/Kg) than sahlia (2.06±0.21 mg/Kg). Similar CAD levels were found in Sardine (Özogul and Özogul 2006), Mackerel (Zhai *et al*., 2012), Herrings (Özogul *et al*., 2002), and Tilapia (Kulawik *et al*., 2013). On the other hand, Yongsawatdigul *et al*. (2004) observed that the Indian Anchovy has greater CAD levels (863 mg/Kg). According to Table 1, the EDI values of CAD (mg/day) varied from 0.100 in Sahlia to 0.396 in Feseikh. If higher quantities of CAD were consumed, it could increase the toxicity of HIS. Furthermore, CAD is linked to intestinal and stomach malignancies (Benkerroum, 2016).

Putrescine

With regard to PUT, the obtained data showed that PUT formed in every salted fish sample that was analyzed. Feseikh has the greatest PUT residues by a large margin, much like CAD. In the salted fish under examination, PUT levels varied from 1.67 to 6.99 mg/kg (Fig. 2B). The PUT residues that were measured in the fish samples analyzed for this inquiry agree with those measured in Sardine (Özogul and Özogul, 2006), Mackerel (Zhai *et al*., 2012), Herrings (Özogul *et al*., 2002), and Tilapia (Kulawik *et al*., 2013). Yongsawatdigul *et al*. (2004) revealed that the Indian Anchovy had comparatively higher PUT levels (259.9 mg/Kg). In salted fish, the EDI values of PUT (mg/day) varied from 0.081 in sahlia to 0.339 in feseikh (Table 1). Because PUT has the least hazardous effects, there is little evidence available about NOAEL or the risk assessment of PUT (Koutsoumanis *et al*., 2010). However, by blocking their oxidative inhibition mechanism, PUT may amplify the harmful effects of TYR and HIS (Bulushi *et al*., 2009). Additionally, PUT is linked to the development of gastrointestinal tumors and neurological disorders (Benkerroum, 2016).

Spermine

The analyzed salted fish samples were subjected to estimations of SPM levels. The findings indicated that sahlia had the lowest average concentrations (1.44±0.03 mg/Kg), whereas feseikh had the greatest SPM content (5.64±0.04 mg/Kg) (Fig. 3A). In Mackerel, SPM was also found at 1.8 mg/Kg (Zhai *et al*., 2012). It is unlikely that SPM was not found in Sardine (Özogul and Özogul, 2006) or Herrings (Özogul *et al*., 2002). Higher SPM levels were found in Indian anchovy (Yongsawatdigul *et al*., 2004) and tuna (Veciana-Nogués *et al*., 1997). The studied fish samples had SPM EDI values (mg/day) ranging from 0.060 in sahlia to 0.273 in feseikh (Table 1). To the best of our knowledge, NOAEL SPM in fish is not well understood or documented. Nonetheless, a number of neurological conditions and neurodegenerative diseases, including cancer, psoriasis, and epilepsy, are linked to SPM (Benkerroum, 2016).

Spermidine

The other polyamine under test in this investigation is SPD. The fish samples under examination had mean concentrations of SPD that were as follows: Salted sardine (4.97±0.07 mg/Kg) > sahlia (3.94±0.06 mg/Kg) > feseikh (5.16±0.06 mg/Kg) (Fig. 3B). These concentrations are consistent with those seen in Sardine (Özogul and Özogul 2006), Mackerel (Zhai *et al*., 2012), Herrings (Özogul *et al*., 2002), and Tilapia (Kulawik *et al*., 2013). For each fish under examination, EDI values were computed for SPD (Table 1). EDI values for salted sardine (mg/day): 0.241, 0.191, and 0.250 in feseikh and sahlia. Like SPM, no information is available regarding NOAEL for SPD in fish. The most powerful carcinogenic N-nitrosamines are precursors of SPD. Moreover, SPD promotes the growth of tumors and is linked to ischemia and cystic fibrosis (Benkerroum, 2016).

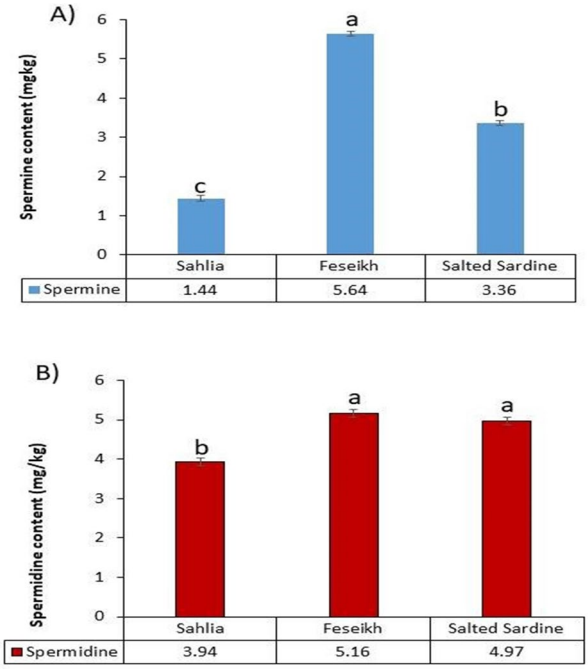

Fig. 3. A) Spermine, B) Spermidine contents (mg/kg) in the examined sahlia, feseikh, and salted sardine. Columns with different letter are significantly different at ($p< 0.05$).

Microbial counts and their correlation with total BAs in fish

Feseikh had the highest level of BAs (172.3±1.42 mg/Kg) > salted sardine (128.15±1.98 mg/Kg) > sahlia (66.51±1.88 mg/Kg), according to a calculation of the total BAs among the fish species investigated (Table 2).

Table 2. Total biogenic amines contents, total microbial counts and their correlations in the examined salted fish samples.

	Sahlia	Feseikh	Salted Sardine
TBA	66.51±1.88^{c}	172.3±1.42^{a}	128.15±1.98^{b}
TPC	3.44±0.02^{b}	5.42±0.01^{a}	3.38±0.01^{b}
TPsC	3.21±0.01^{b}	4.54±0.02^{a}	2.87±0.02^{b}
TSC	2.21±0.01^{c}	3.54±0.02^{b}	4.23±0.01^{a}
r	0.66	0.75	0.68
p	0.01	0.01	0.01

The high concentration of the corresponding free amino acids in the salted fish species, including them, is most likely the cause of their high level of total BAs (Biji *et al*., 2016). Furthermore, feseikh is a type of fermented fish that is cooked and sold by neighborhood feseikh stores in Egypt. The likelihood of the fish spoiling quickly due to microbial decomposition is significant (Visciano *et al*., 2014). As a result, we expanded this research to look at the fish species under investigation's microbiological status as well as the correlation between the total BAs and the microbial counts. Faseikh was found to have the greatest TPC (5.42±0.01 $\log_{10}$ cfu/g), with salted sardine coming in second (3.38±0.01 $\log_{10}$ cfu/g) and sahlia third (3.44±0.02 $\log_{10}$ cfu/g). Comparable to the TPC were the TPsC and TSC (Table 2). The TPC counts for tuna (Jääskeläinen *et al*., 2019) and tilapia (Kulawik *et al*., 2013) were almost identical. It's interesting to note that in the various fish species, TBAs and TPC showed strong positive connections (Table 2). Similar positive associations have been shown in fish (Visciano *et al*., 2014), camel meat (Tang *et al*., 2020), and cheese (Ma *et al*., 2020) between microbial numbers and the development of BAs. These findings strongly imply that the degree of BA production in raw fish is dependent on the type of fish, storage circumstances, and initial microbial populations. As a result, it is crucial to adhere to industry standards while processing and manufacturing fish, which include limiting microbiological contamination, handling fish carefully, and making sure the fish is chilled to no more than 4°C (FDA, 2011).

CONCLUSION

The study's findings showed that several BAs were found in salted fish that was sold in Egypt. Feseikh exhibited the greatest microbiological populations and total BAs. The Egyptian populace did not pose any risk, according to the calculation of the EDI values of the studied BAs through fish consumption. On the other hand, consuming large amounts of salted fish contaminated with BAs-specifically, TYR and HIS-may pose a major risk to one's health, especially scombroid toxicity. As a result, Egypt should process, store, distribute, and market fish using effective hygienic practices.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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