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Spermidine and Tyramine Profiling in Fish: Insights into Biogenic Amines Dynamics

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ABSTRACT: Biogenic amines are undesirable compounds formed through the decarboxylation of free amino acids by pathogenic microbes, They pose serious risks to public health and food safety. These amines serve as critical biomarkers for evaluating food quality and safety. Their physiological implications for human health necessitate vigilant monitoring, particularly in fish, where elevated concentrations can cause histamine poisoning and toxicity. This study employed liquid chromatography–electrospray ionization–tandem mass spectrometry (LC-ESI-MS/MS) to quantify spermidine and tyramine levels in Mackerel fish. Twenty random samples, comprising 10 frozen and 10 grilled, were collected from various markets and restaurants in the New Valley Governorate, Egypt. The analysis revealed that mean values of spermidine concentrations (mg/100g) were 233.2 ± 25.5 in raw frozen fish and 186.80 ±10.7 in grilled fish. The average concentrations of tyramine were 1.22 ± 0.09 in raw frozen fish and 3.54 ± 0.3 in grilled fish. Based on the established permissible limits recommended by EOS, all samples of frozen mackerel fish 100% and 90% of grilled fish were exceed the permissible spermidine limits. However, all frozen and grilled fish samples were within acceptable tyramine levels.

KEYWORDS: Biogenic amines, Spermidine, Tyramine, Mackerel fish, LC-ESI-MS/MS

1. Introduction

Fish is a significant component of the global diet, offering essential nutrients such as proteins and polyunsaturated fatty acids crucial for sustaining life processes and providing energy [1]. Fish is susceptible to rapid protein degradation due to bacterial enzymatic activity, leading to the production of biogenic amines (BAs). These compounds serve as important quality indicators and tools for evaluating food safety [2] . Mackerel, an important species of the Scombridae family, is notable for its high concentrations of free amino acids in its muscle fibers, which predisposes it to elevated levels of biogenic amines [3] . Biogenic amines are low-molecular-weight organic bases with biological activity. They are formed in fish by the decarboxylation of amino acids by microorganisms or the transamination of aldehydes and ketones by amino acid transaminases [4] . Regulatory agencies like the FDA and EFSA monitor the presence of BAs in food due to their toxicological effects on human health. If you take too much, you might have bad reactions, especially if you are sensitive, have a disease that affects your digestive system, or are taking monoamine oxidase inhibitors (MAOIs) or diamine oxidase inhibitors (DAOIs), which are drugs used to treat depression, allergies, malaria, and other mental health issues [5]. The presence of significant quantities of biogenic amines in food is a public health concern due to their toxicological and physiological effects. Their formation is contingent upon a variety of factors, such as the purity of raw materials, the concentration of amino acids, bacterial activity, and environmental conditions [6, 7]. Biogenic amines can be classified as heterocyclic (e.g., histamine and tryptamine), aliphatic (e.g., putrescine and cadaverine) and aromatic (e.g., tyramine and phenylethylamine) according to their chemical structures. These compounds can also be grouped as monoamines (e.g., tyramine and phenylethylamine) and diamines (e.g., histamine, putrescine and cadaverine) based on the number of amine groups in the compound of the biogenic amines [8].

Based on their chemical structure and variety of amine groups, spermidine is a well-known polyamine that is generated from putrescine and is essential for controlling cellular processes and preventing oxidative damage [9]. Tyramine is a trace monoamine is synthesized from the amino acid tyrosine by tyrosine decarboxylase [10]. Spermidine has the potential to exacerbate histamine toxicity in fish tissue by inhibiting intestinal histamine-metabolizing enzymes. This phenomenon may result in symptoms such as gastrointestinal discomfort, edema, and cutaneous reddening [11]. Furthermore, the reactivity of spermidine with nitrites during cooking can produce carcinogenic nitrosamines, emphasizing the necessity of monitoring BA levels, especially in grilled fish, where the creation of hazardous compounds is amplified [12, 13] . Tyramine, on the other hand, causes the adrenal medulla and central nervous system to release catecholamines, which in turn causes respiratory problems, migraines, vomiting, nausea, elevated heart rate and blood pressure, hypertensive crisis, and neurological conditions like schizophrenia, Parkinson's disease, and Reye's syndrome [14]. It is essential to comprehend the concentrations of these amines in frequently ingested fish products to evaluate food safety and safeguard public health [15]. The primary aim of this study is to conduct a comprehensive analysis of biogenic amine concentrations, spermidine and tyramine, in frozen and grilled mackerel fish obtained from various local shops and restaurants in the New Valley governorate, Egypt.

2. Materials and Methods

2.1. Collection of the samples and Preparation

Twenty random Mackerel fish samples preserved at, comprising 10 frozen fish -18°C and 10 grilled fish at 50-60°C, were procured from various local shops and restaurants in the New Valley Governorate, Egypt, for spermidine and tyramine levels assessment. Each sample underwent standardized procedures of gutting, skinning, and slicing before homogenization to ensure a uniform composition. The homogenized samples were subsequently stored at -18°C until analysis, adhering to the wet weight protocol established by [16].

2.2. Apparatus

The analysis of the sample was performed at The National Research Center, Dokki, Cairo, Egypt by using liquid chromatography–electrospray ionization–tandem mass spectrometry (LC-ESI-MS/MS) with an Exion LC AC system for separation and SCIEX Triple Quad 5500+ MS/MS system equipped with an electrospray ionization (ESI) for detection. Data processing was performed using SCIEX OS Version 1.6.1.29803. The results were expressed as mg/kg wet weight of sample.

2.3. Standards and reagents provided by [17].

LC-MS-grade solvents: trichloroacetic acid (TCA), 0.1% formic acid, Ultra-pure water, Methanol and reagent: Spermidine trihydrochloride (C7H19N3, CAS No. 124-20-9, ≥ 98%).

2.4. Extraction method

Biogenic amines were extracted from the samples using the techniques provided by [17]. For the analysis, a representative portion of fish tissues (50 g) was chopped into small pieces and finely ground with a blender to homogenise it before extraction. A total of 5g of fresh/frozen fish sample was homogenised for one minute with 15mL of TCA 5% using an Ultra-Turrax S 18 N-10 G (IKA-Werke Gmbh & Co., Germany). The obtained homogenate was decanted into centrifuge tubes and spun at 2500g for 10 min at 4°C. After removing the extracts, 10mL of TCA 5% was added to the remaining solid and the process was repeated. Then, both extracts were combined and collected in a plastic vial. For liquid chromatography and fluorescence detection (LC-FD), the derivatisation process with dansyl chloride 1 mL of supernatant TCA extract was derivatised by adding 300 mL of NaHCO3 (saturated solution), 200 mL of NaOH2 N and 2 mL of dansyl chloride solution (10 mg/mL in acetone, daily prepared). The mixture was left under magnetic stirring in darkness for 45 min at 45°C. Then, it was allowed to cool at room temperature, and 100 mL of NH4OH 28% were added for neutralizing the excess of dansyl chloride. Finally, 400 mL of acetone were added to the solution before LC-FD analysis For LC-MS/MS, the STRATA X cartridge were conditioned with 4

mL of methanol followed by 4 mL of Milli-Q water using a vacuum system. Then, 2 mL of the sample with a pH adjusted to 11 with NH4OH 28% were passed through the cartridges at a flow rate of approximately 1 mL/min. After sample loading was complete, sample flasks and cartridges were rinsed with 2 mL of a mixture of MeOH/H2O (5:95, v/v). Cartridges were, then, dried under vacuum for 5 min, to remove excess of water. Analytes were eluted from the STRATA X sorbents with 2 + 2 mL of a mixture methanol/ acetic acid (99:1, v/v). The eluting solutions were dried with nitrogen gas, the residue dissolved in 2 mL of HCl 0.1 M, filtered and injected in LC-MS/MS.

2.5. LC-MS analysis

It was developed according to [18] . The LC separation was performed using an Alliance 2695 HPLC separation module (Waters). In positive ion (PI) mode, a column Synergi Hydro (250 _ 4.0 mm I.D., 5 lm) from Phenomenex (Torrance, CA, USA) was used. The mobile phase for LC–MS/MS analysis was ammonium formate 15 mM and formic acid in water (pH 3.3) (A) and methanol (B), at a flow rate of 0.5 mL min_1.The gradient program was: 0 min 30% B, 0–15 min 90% B, 15–20 min 30% B, 20–25 min 30% B. The injection volume was 20 mL. The tandem MS analyses were performed on a Micromass Quattro triple quadrupole mass spectrometer (Manchester, UK). Instrument control, data acquisition and evaluation were done with the Masslynx NT software (v. 3.4). The applied parameters were: radio frequency lens, 0.2 V; electrospray source block, 150 _C; low mass (LM) 1 resolution, 12.0; high mass (HM) 1 resolution, 12.0; LM 2 resolution, 12.0; HM 2 resolution, 12.0; multiplier 650 V; desolvation temperature: 350◦ C; argon collision gas 2.5x10-3 mbar; cone nitrogen gas flow, 50 L h_1; desolvation gas: 500 L h_1. In PI mode, the extractor voltage was 3.0 V and capillary voltage 3.5 kV. Optimisation of electrospray (ESI) interface parameters were performance by directly infusing a standard solution into the LC - MS/MS system at a flow rate of 20 lL min_1. Full-scan analyses and MS/MS product ion scan mass spectra of the selected ion were performed over a scan range of m/z 50-225 using a step size of 0.1 Da and a rate of 0.2 scan/s. The optimal quantification and confirmation transitions, their respective cone voltages, collision energies and time window are listed in Table 1. The dwell time and the inter channel delay were set at 0.2 and 0.01 s, respectively.

Mobile phase

The separation was performed using Kinetex® F5 100Å LC Column (4.6×100 mm, 2.6 µm). The mobile phases consisted of two eluents A: 0.1% formic acid in water; B: 0.1% formic acid in methanol (LC grade). The mobile phase was programmed as following, 20% B at 0 min, 20-95% B from 0-3 min, 95% B from 3-5 min, 98-20% B from 5-5.01 min. and finally 20% B from 5.01-7 min The flow rate was 0.6 ml/min and the injection volume was 5 µl. For MRM analysis of the selected biogenic amines, positive ionization modes were applied with the following parameters: curtain gas: 25 psi; Ion Spray voltage: 5500; source temperature: 550°C; ion source gas 1 and 2 were 55 psi.

2.6. Statistical analysis

The data was analyzed using one-way ANOVA, with a significance level of $P < 0.05$. The mean values and standard errors of the mean were conducted using SPSS Version 12.0 for Windows (SPSS Inc., Chicago, IL, USA).

3. Results

Table 1: Statistical analytical values of spermidine in the examined samples of Mackerel fish (mg/100g).

Mackerel fish Samples	No.	Min.	Max.	Mean±SE
Raw	10	87.46	379.00	233.20±25.50[a]
Grilled	10	2.25	602.60	186.80±10.70[b]

Means with different superscripts are significantly different (P<0.05)

3.1. Biogenic amines in frozen fish

Spermidine were found to be present in high ranges, it was detected in raw fish samples with a range from 87.46 mg/100g to 379.00 mg/100g with an average 233.2 ± 25.5 mg/100g. The obtained results are shown in Table 1 . the

Table 2: Acceptability of the examined samples of mackerel fish based on their levels of spermidine according to EOS, (2010)

Mackerel fish samples	MRL (mg/100g)	Accepted samples		Unaccepted samples	
		NO	%	NO	%
Raw frozen	20	0	0	10	100
Grilled	20	1	10	9	90

(MRL) Maximum Residual limits of spermidine levels in fish are 20mg%(EOS, 2010)

Table 3: Statistical analytical values of tyramine in the examined samples of Mackerel fish (mg/100g).

Mackerel fish samples	No.	Min.	Max.	Mean±S.E
Raw frozen	10	0.36	6.81	1.22 ± 0.09^{a}
Grilled	10	0.85	6.59	3.54 ± 0.3^{b}

Means with different superscripts are significantly different (P<0.05)

results achieved in Table 3 show tyramine concentrations were 1.22 ± 0.09 mg/100g in raw fish with a range from 0.36 mg/100g to 6.81 mg/100g.

3.2. Biogenic amines in grilled fish

The findings outlined in Table 1 revealed that Spermidine level ranged from 2.25 mg/100g to 602.6 mg/100g in grilled mackerel fish with an average 186.80±10.7 mg/100g. Data recorded in Table 3, it was obvious that the tyramine levels were 3.54 ± 0.3 mg/100g in grilled mackerel fish within range from 0.85mg/100g to 6.59 mg/100g.The results gained in Table 2 showed that the examined samples of mackerel fish were all unaccepted and exceeded the allowable limits of spermidine levels according to the permissible limits recommended by (18) (20 mg/100g) while most of grilled fish were unaccepted (90%). While, the obtained results in Table 4 revealed that 100 % of all samples of frozen and the grilled fish were accepted based on their tyramine levels.

Table 4: Acceptability of the examined samples of mackerel fish based on their levels of tyramine according to EOS, (2010).

Mackerel fish samples	MRL (mg/100g)	Accepted samples		Unaccepted samples	
		NO	%	NO	%
Raw frozen	20	10	100%	0	0
Grilled	20	10	100%	0	0

(MRL) Maximum Residual limits of tyramine levels in fish are 20mg% (EOS, 2010).

Discussion

Biogenic amines, as biologically active compounds, present a potential health risk when consumed in high quantities, as their toxicity can lead to various foodborne illnesses. As a result, biogenic amine analysis is critical since it serves as both an indicator of food freshness and decomposition as well as a critical factor for guaranteeing food safety. For dietary items like fish, where biogenic amine buildup is more likely, this monitoring is especially crucial [19]. Two factors spark interest in the study of BAs in food: the possibility of using BAs as indicators of food quality and their potential toxicity. BAs could be a sign of microbial contamination and poor handling or processing cleanliness [20]. BAs is a quality and safety indicator that is formed by various factors such as improper harvesting techniques, improper handling and other vessel operations, post-catching contamination, insufficient chilling, and temperature abuse [21]. Spermidine, a polyamine molecule with the scientific name N-(3-aminopropyl)-1,4-butanediamine, is one of the most important of these biogenic amines. Spermidine, predominantly produced by microbial activity, acts as a biological biomarker for evaluating food quality [22, 23]. The level of oral toxicity of spermidine does not exceed 600 mg/kg, as reported by [24]. Interestingly, raw fish had a higher mean quantity of spermidine contamination than grilled fish. We can attribute this to the aseptic role of smoke, which inhibits the growth of amine-decarboxylating bacteria [25]. According to the recorded spermidine results for the examined Mackerel fish, most of the results in this study were higher than permissible limits determined by [26, 27]. This gap could be related to spermidine's quick metabolic changes [28]. Additionally, exposing fish to high temperatures while cooking may accelerate the development of biogenic amines. Discrepancies in spermidine concentrations across different studies suggest that variability is influenced by factors such as storage conditions and bacterial activity [4, 29]. Factors such as raw material quality and handling techniques during shelf life likely contribute to increased microbial contamination and bacterial decarboxylase action, leading to the higher quantities of spermidine reported in samples [30]. The lowest results of spermidine levels in raw frozen fish were

recorded by [31, 32, 4, 33] who recorded that the mean value of spermidine is 104 ± 5.2, 06.2±0.41, 4.6±0.38 and 112.6± 0.5 mg/100g, respectively. [28] showed concentration of spermidine ranged from 105.8 to 132 mg/100g in mackerel fish frozen at 0°C. The precise control of freezing temperatures can significantly influence the levels of spermidine by limiting microbial and enzymatic activities. Because of their high contents, biogenic amine concentrations can be considered chemical indicators for fish spoilage. BAs concentrations significantly indicate mishandling, and poor hygienic conditions during the processing and consumption of food containing high amounts of tyramine can have toxic effects and become one of the most important food safety issues, as tyramine toxicity illustrates [34]. Moreover, tyramine, when exposed to high temperatures, formed secondary amines that combined with nitrites, consequently forming nitrosamine, which is believed to be one of the main carcinogens [35]. The unfavorable processing temperature and the presence of additives like pepper, tomatoes, and other spices, which play a significant role in the growth and multiplication of such biogenic amine-forming microorganisms, may be to blame for the higher level of tyramine in the grilled fish than in the frozen fish [36]. Tyramine levels were higher in grilled fish than raw fish which may be attributed to presence of higher temperature which favored proteolytic and decarboxylase activities of microorganisms resulting in increased tyramine concentrations in these food articles containing higher contents of tyrosine (15). US Food and Drug Administration and European Food Safety Authority had established a tolerance level of tyramine in fish of 100 mg/kg [26, 27].The majority of fish samples were found to have far lower levels of BAs than the unaccepted standards; therefore they can be regarded safe for human consumption. There was 1.2 mg/100g of tyramine in the frozen fish that was tested, which was similar to the results found by [37] for frozen mackerel fish samples. These results were also similar to those found by [32] for frozen mackerel fish, which were 1.003 ± 0.13 mg/100g. While higher results were revealed in local and imported canned mackerel with a mean value of 11.12 ± 0.040 and 9.94 ± 0.058 mg/100g, respectively [38]. Although Canning involves processes such as heating, which can kill or inhibit certain microbes but may also cause the release or formation of biogenic amines like tyramine from the breakdown of proteins and other compounds in the fish [5] . So, the levels of tyramine found in the mackerel fish samples were lower than those found by [33] in the chub mackerel samples, where the mean value was 130.8 mg/100 g, and [4] in the frozen mackerel samples, where the mean value was 3.13 mg/100 g. They were also much lower than the highest levels allowed by [26, 27] at 100 mg/kg (10 mg/100 g). Effective handling and storage techniques, including rapid freezing and maintaining low temperatures, can inhibit microbial growth and enzymatic activities that contribute to tyramine formation. Studies highlight the importance of these practices, highlighting the crucial role of proper storage conditions in controlling biogenic amine levels [39]. Tyramine may be toxic if consumed at 100–800 mg/kg. A high intake of Tyramine can cause adverse toxicological effects and diseases such as high blood pressure, brain hemorrhage, palpitations, nausea, and diarrhea [40].The quality criteria with respect to the presence of BAs vary from food to food, as the existence of potential effectors dramatically influences the toxic thresholds of BAs in foods. Generally, we consider an intake of over 40 mg of BAs per meal as potentially harmful, and we expect an elicitation of over 1000 mg as toxic [3]. The concentration of the biogenic amines tyramine and spermidine not only serves as a valuable indicator of spoilage, but also underscores the significance of post-processing and post-harvest handling practices in preserving food quality [41].Its presence in all Mackerel samples is notable, emphasizing its potential as both a quality indicator and a source of toxicological concern, as it may have surpassed the recommended safe allowable limits specified by the Egyptian Organization for Standardization [42] . Regulating them is necessary to either prevent or limit them, while also enhancing fish sanitation. Proper cooling and handling procedures are

essential for reducing histamine development and ensuring the safety and quality of fish products.

Conclusion

According to the current research, spermidine levels were higher in frozen mackerel than in grilled mackerel. On the other hand, when compared to frozen fish, grilled mackerel fish showed noticeably greater tyramine contents. These results highlight the need for strict quality control and monitoring protocols to guarantee the safety of mackerel fish for human consumption.

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