Estimation of biogenic amines in salted – fermented fish and some fish products in Cairo Markets with special references to its storage

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Abstract: The main objective of the present study is to analyze major biogenic amines in Feseekh and sardine (salted) and other some commercial fish products to determine the concentrations of biogenic amine during different storage temperatures and durations. Seven different kinds of fish product samples, according to the type and part of fish used for preparation, were purchased at the local department stores and transported in ice to the laboratory. Samples were analyzed for biogenic amine content. All samples were purchased and stored at three different temperatures, 0, 5, 10, 15 and 20 °C. The concentration of biogenic amines was determined after 20 days and at 40 and 60 days of storage. Extraction of the samples and HPLC determination of biogenic amines were carried out. In summary, most commercial fish product samples contained only trace amounts (below 200 mg/kg) of biogenic amines, and the concentrations did not change significantly during various storage conditions, indicating that these samples are safe for human consumption. However, further studies are required to control the concentrations of biogenic amines under proper storage condition, since the contents of biogenic amines tend to increase in the concentration as the storage temperature increases. The results showed that the free amino acid and biogenic amines content of Feseekh fish high and low salt and other fish products increased significantly during long time of ripening and storage. So it could be concluded that salted-fermented fish (Feseekh, sardine and meloha) can be consumed without any health risks between 20 and 40 days, but it could be hazardous after 60 days, due to the increased free amino acids and biogenic amine content.

Key words: Biogenic amines- storage temperatures- HPLC- Feseekh- Sardine- Meloha- Duration.

1. Introduction

Biogenic amines are naturally occurring, low molecular weight organic bases, ubiquitous in animals and plants. Putrescine, cadaverine, spermidine and spermine have an aliphatic structure, histamine and tryptamine have a heterocyclic structure and tyramine and phenylethylamine have an aromatic structure. The formation of high levels of biogenic amines, especially histamine, in fish products may be rapid, and their development depends on the number of microorganisms present. Biogenic amines, can be produced and degraded by normal metabolic activities in animals, plants and microorganisms. These amines are mainly produced by microbial decarboxylation amino acids in foods (Brink et al., 1990; Hala’sz et al., 1994). Typical symptoms of biogenic amine intoxication in human can be nausea, respiratory distress, hot flushes, sweating, heart palpitation, headache, bright red rash, oral burning, and hypertension as well as hypotension (Rice, Eitenmiller & Koehler, 1976).

Askar and Treptow (1993) have suggested histamine at a concentration of 500 mg/kg to be hazardous for human health. On the other hand, Brink et al. (1990) reported that 100–800 mg/kg of tyramine and 30 mg/kg phenylethylamine in foods are toxic. Putrescine and cadaverine inhibit intestinal diamine oxidase and histamine-N-methyltransferase which metabolize histamine, resulting in an increase of histamine toxicity (Stratton et al., 1991). Furthermore, putrescine (Bills et al., 1973), cadaverine (Warthesen et al., 1975), spermidine (Bills et al., 1973; Smith, 1980), spermine and agmatine (Smith, 1980) are reported to be potentially carcinogenic by converting to nitrosamine. Tyramine has also been identified as the major mutagenic precursor in animal (Ochiai et al., 1984).

Feseekh and sardine (salted) are the traditional name for the salted-fermented fish produced in Egypt. It is popular not only as an appetiser, but also as the main dish in spring and some feasts in Egypt (El-Sebaiy & Metwalli, 1989). The fermented–salted fish products contain relatively high amounts of amino acids, the
Degradation products of fish protein. Since the product produced by a small-scale fishery manufacturer by a traditional primitive method and also the low-salt produced products are stable only for a limited period, these products could be potentially the source for biogenic amine. The main objective of the present study is to analyze major biogenic amines in Feseekh and sardine (salted) and some commercial fish products to determine the concentrations of biogenic amine during different storage tempratures and durations.

2. Materials and Methods:

Samples:

Seven different kinds of fish product samples, according to the type and part of fish used for preparation, were purchased at the local department stores and transported in ice to the laboratory. The types of samples were as follows: and analyzed for biogenic amine content. All samples were purchased and stored at three different temperatures, 0, 5, 10, 15 and 20 °C. Bouri fish was purchased. The fish were cleaned thoroughly and left at room temperature for 24 h until it loses its water and acquired the right degree of expansion a then put it in a glass or plastic jar, with plenty of salt between the layers of gill lamellae. It was then tightly sealed and left for 60 days at room temperature. The concentration of biogenic amines was determined after 20 days and at 40 and 60 days of storage.

Preparation of Standard Amine Solutions:

Extraction of the samples and HPLC determination of biogenic amines were carried out according to the procedure described by Ben-Gigirey, de Sousa, Villa, and Barros-Velazquez (1998). The detection limits were approximately 4 mg/kg for putrescine, histamine, tyramine and spermine, and 6 mg/kg for cadaverine and spermidine. Stock solutions of putrescine, cadaverine, spermidine and spermine were prepared at a concentration of 10,000 mg/l distilled water, and diluted to 100 or 1000 mg/l for working solutions.

Derivatization of Sample Extracts:

One millilitre of each sample extracted was mixed with 200 µl of 2 M sodium hydroxide and 300 µl of saturated sodium bicarbonate. Two milliliters of a dansyl chloride solution (10 mg/ml) prepared in acetone were added to the mixture, and then incubated at 40 °C for 45 min. Residual dansyl chloride was removed by the addition of 100 µl of 25% ammonium hydroxide. After 30 min incubation at room temperature, the extract was adjusted to 5 ml with acetonitrile. Finally, the mixture was centrifuged at 2500 xg for 5 min and the supernatant was filtered through 0.2 µm-pore-size filters (Millipore Co., Bedford, MA). Biogenic amine standard solutions were diluted to 10 ml with 0.4 M perchloric acid to obtain the range of 2 mg/kg to 100 mg/kg. These solutions were derivatized using the same method in the sample extracts to obtain a calibration curve.

Extraction of Amino Acids and Biogenic Amines:

The amino acids and biogenic amines in the Feseekh samples were extracted using the method described by Simon-Sarkadi and Holzpfel (1994). To extract biogenic amines, 10 ml of 10% trichloroacetic acid (TCA) was added to 3 g of samples of fish products the mixture was shaken for 1 h using a shaker (Gerhardt Ls 500i, Germany). The extract was filtered through Whatman filter paper. To remove the fat content, the samples were kept at -20 °C for one day, and then centrifuged (MLW, T 24, Germany) at 7000g for 15 min. Supernatants were collected and filtered through a 0.25 µm membrane filter.

Determination of Amino Acids and Biogenic Amines:

The analysis of amino acids and biogenic amines was performed with an AAA 400 amino acid analyser (Ingos Ltd., Czech Republic) equipped with a Watrex Polymer 8 ion-exchange column (20 x 3.7 cm) for amino acids and an Ostion LG ANB ion-exchange column (6 x 3.7 cm) for biogenic amines. Free amino acids and biogenic amines were separated by stepwise gradient elution using Li+/K+ buffer systems for amino acids and using Na+/K+ buffer system for biogenic amines. Colorimetric detection was accomplished at 570 and 440 nm after post-column derivatisation with ninhydrin reagent (Csomos & Simon-Sarkadi, 2002). The analyses were done in duplicate. The average values ± The relative standard deviations.

3. Results and Discussion:

The study was carried out to investigate and survey the amount and concentrations of biogenic amines in some fish products with special attention to some salted fish products like sardine, meloha and feseekh (high and low salt). The study was included seven Egyptian fish products; canned fish (42), fish flea (17),...
smoked fish (53), sardine (16), salted fish (meloha) (27), feseekh (low salt) (36) and feseekh (high salt) (24). The study revealed that the highest concentration of biogenic amines was found in feseekh (low salt) (1799 mg/kg) followed by feseekh (high salt) (1078 mg/kg), followed by smoked (282 mg/kg) on the other hand the lowest concentration of biogenic amines was found in canned fish (18 mg/kg). The highest concentration was for histamine while the lowest concentration was for spermidine. The amounts of histamine, cadaverine, tyramine and putermine were high reaching to 1026 mg/kg, 912 mg/kg and 657 mg/kg respectively while speridine and spermine contents were determined to be 249 and 219 mg/kg respectively in case of feseekh (low salt) relatively high levels of biogenic amines were detected 1799 mg/kg.

### Table 1: Biogenic amines contents in some fish products (mg/kgDw)

<table>
<thead>
<tr>
<th>Fish products</th>
<th>Cadaverine</th>
<th>putresine</th>
<th>Histamine</th>
<th>Tyramine</th>
<th>Spermidine</th>
<th>Spermine</th>
<th>Total conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canned fish (42)</td>
<td>0</td>
<td>1±0.05</td>
<td>3±0.15</td>
<td>2±0.1</td>
<td>7±0.35</td>
<td>5±0.25</td>
<td>0</td>
</tr>
<tr>
<td>Fish flea (17)</td>
<td>0</td>
<td>20±1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>20±1</td>
</tr>
<tr>
<td>Smoked fish (53)</td>
<td>85±4.25</td>
<td>136±6.8</td>
<td>0</td>
<td>14±0.7</td>
<td>14±0.69</td>
<td>33±1.65</td>
<td>282±14.1</td>
</tr>
<tr>
<td>Sardeen (salted) (16)</td>
<td>13±0.65</td>
<td>30±1.5</td>
<td>45±2.2</td>
<td>244±12.2</td>
<td>43±2.1</td>
<td>25±1.2</td>
<td>180±9.1</td>
</tr>
<tr>
<td>Salted fish (meloha) (27)</td>
<td>20±1.1</td>
<td>20±1</td>
<td>57±2.9</td>
<td>12±0.6</td>
<td>32±1.6</td>
<td>16±0.8</td>
<td>157±7.8</td>
</tr>
<tr>
<td>Feseekh (low salt) (36)</td>
<td>665±33.2</td>
<td>210±10.5</td>
<td>579±18.9</td>
<td>171±8.6</td>
<td>55±2.7</td>
<td>119±5.9</td>
<td>1799±89.9</td>
</tr>
<tr>
<td>Feseekh (high salt) (24)</td>
<td>129±6.9</td>
<td>241±12.05</td>
<td>345±12.3</td>
<td>233±11.6</td>
<td>75±3.7</td>
<td>55±2.7</td>
<td>1078±53.9</td>
</tr>
<tr>
<td>Total conc.</td>
<td>912±40.6</td>
<td>657±32.9</td>
<td>1026±51.3</td>
<td>674±33.7</td>
<td>219±10.9</td>
<td>249±12.5</td>
<td>3516±74.3</td>
</tr>
</tbody>
</table>

Mean of the samples number ± standard error

### Table 2: Changes in different biogenic amines concentration in fish products during storage at different temperatures (mg/kg Dw)

<table>
<thead>
<tr>
<th>Biogenic amines</th>
<th>0 °C</th>
<th>5°C</th>
<th>10°C</th>
<th>15°C</th>
<th>20 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cadaverine</td>
<td>29±1.4</td>
<td>73±3.6</td>
<td>125±6.3</td>
<td>250±12.5</td>
<td>366±18.3</td>
</tr>
<tr>
<td>putresine</td>
<td>22±1.1</td>
<td>613±30.6</td>
<td>753±37.6</td>
<td>815±40.8</td>
<td>1230±61.5</td>
</tr>
<tr>
<td>Histamine</td>
<td>27±1.4</td>
<td>253±12.7</td>
<td>245±12.3</td>
<td>518±25.9</td>
<td>811±40.6</td>
</tr>
<tr>
<td>Tyramine</td>
<td>15±0.8</td>
<td>133±6.7</td>
<td>205±10.3</td>
<td>225±11.3</td>
<td>263±13.2</td>
</tr>
<tr>
<td>Spermidine</td>
<td>0</td>
<td>0</td>
<td>3±0.15</td>
<td>25±1.3</td>
<td></td>
</tr>
<tr>
<td>Spermine</td>
<td>2±0.1</td>
<td>105±5.3</td>
<td>158±7.9</td>
<td>177±8.9</td>
<td>187±9.4</td>
</tr>
</tbody>
</table>

Mean of the samples number±standard error
Changes in the concentrations of various biogenic amines in some Egyptian fish products samples during storage duration for 20 days, 40 days and 60 days are shown in Table 2. Concentration of cadaverine increased from 53 mg/kg to 1030 mg/kg after storage for 60 days, whereas that of histamine from 35 mg/kg to 224 mg/kg during and after 60 days of storage. Putrescine contents increased from 22 mg/kg to 240 mg/kg after the storage for 60 days. While spermine and spermidine contents did not change much during the storage (43 and 11 mg/kg, respectively), except tyramine with a slight increase (167 mg/kg after 60 days).

The content of putrescine was 22 mg/kg initially and increased to 815 mg/kg after the storage at 15°C and for 1230 mg/kg at 20°C. Table 3 shows the biogenic amine contents in fish sample. Spermine contents increased from 2 to 187 mg/kg at 20°C. Concentrations of histamine, cadaverine and tyramine in the samples were 811 mg/kg, 366 mg/kg and 263 mg/kg at 20°C.

The decrease in cadaverine, histamine and spermidine levels may be observed behind 60 days after storage. A similar decrease in the histamine level during the ripening of salted anchovies was reported by Hernández-Herrero, et al., (1999). The authors suggested that a part of the amine content might have been diffused into the brine with other nitrogen fractions during the process of ripening.

A hazard level of histamine for human health has been suggested to be 500 mg/kg (Askar & Treptow, 1993). Putrescine and cadaverine can increase histamine toxicity (Stratton et al., 1991). Moreover, putrescine, cadaverine, spermidine and spermine have also been implicated to be the potential precursors for the formation of nitrosamines (Bills et al., 1973; Smith, 1980; Warthesen et al., 1975). While the histamine content varied between 518 and 811 mg/kg at 15°C and 20°C in the commercial fish product samples, cadaverine and putrescine levels were detected up to 366 and 1230 mg/kg, at 20°C, therefore, might not be completely safe for human consumption under certain storage conditions at high temperature ( 10, 20 and 20°C ).

Furthermore, histamine content in the fish samples during the storage increased to the level of nearly 1000 mg/kg which is considered to be critical to induce histamine poisoning (Askar & Treptow, 1993). Significant increments of cadaverine and spermidine might be also potentially hazardous for health, because inactivation of histamine metabolizing enzymes by biogenic diamines may yield nitrosamines (Bills et al., 1973; Smith, 1980; Stratton et al., 1991). Overall biogenic amine contents appeared to increase during storage at high temperatures especially putrescine showed even higher increase up to 1230 mg/kg, respectively. It is therefore, important to investigate the possibility that biogenic poly-amines could be converted into nitroso compounds. Salted-fermented fish (Feseekh) contained six biogenic amines, namely, histamine, tyramine, putrescine, cadaverine, spermidine, and spermine. The total concentration of biogenic amines increased around 12-fold during the ripening period (130 mg/kg), reaching a concentration of 1615 mg/kg after 60 days of storage.

In summary, most commercial fish product samples contained only trace amounts (below 200 mg/kg) of biogenic amines, and the concentrations did not change significantly during various storage conditions, indicating that these samples are safe for human consumption. However, further studies are required to control the concentrations of biogenic amines under proper storage condition, since the contents of biogenic amines tend to increase in the concentration as the storage temperature increases. The results showed that the free amino acid and biogenic amines content of Feseekh fish high and low salt and other fish products increased significantly during long time of ripening and storage. So it could be concluded that salted-fermented fish (Feseekh, sardine and meloha) can be consumed without any health risks between 20 and 40 days, but it could be hazardous.

Table 3: Changes in biogenic amines concentration in relation to duration time of fish products storage (mg/kg Dw)

<table>
<thead>
<tr>
<th>Biogenic amines</th>
<th>Start</th>
<th>20 Day</th>
<th>40 Day</th>
<th>60 Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cadaverine</td>
<td>53±2.6</td>
<td>411±20.6</td>
<td>715±35.7</td>
<td>1030±51.5</td>
</tr>
<tr>
<td>putrescine</td>
<td>22±1.1</td>
<td>65±3.3</td>
<td>193±9.6</td>
<td>240±12</td>
</tr>
<tr>
<td>Histamine</td>
<td>35±1.7</td>
<td>67±3.4</td>
<td>82±4.1</td>
<td>224±11.2</td>
</tr>
<tr>
<td>Tyramine</td>
<td>15±0.8</td>
<td>27±1.3</td>
<td>42±2.1</td>
<td>167±8.4</td>
</tr>
<tr>
<td>Spermidine</td>
<td>3±0.15</td>
<td>2±0.1</td>
<td>7±0.35</td>
<td>11±0.5</td>
</tr>
<tr>
<td>Spermine</td>
<td>2±0.1</td>
<td>3±0.15</td>
<td>4±0.2</td>
<td>43±2.2</td>
</tr>
</tbody>
</table>

Mean of the samples number ± standard error
after 60 days, due to the increased free amino acids and biogenic amine content.

References:
forming bacteria after the ice storage offish and shrimp. Food Microbiology, 19, 617–625.


