PHYSICOCHEMICAL AND MICROBIOLOGICAL PROPERTIES OF SALTED FERMENTED FISH (FESEEKH) CONSUMED IN EGYPT

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ABSTRACT: The salted fermented fish (Mugil cephalus) Feseekh is not only popular as an appetizer; but also it's popular in some festivities in Egypt as a main dish. Biogenic amines (BA) are known as toxic in food. This study was conducted to investigate biogenic amines (BAs), physicochemical properties, and some microorganisms in salted fermented fish (Feseekh). Fifteen samples of Feseekh samples were obtained from different Egyptian local markets in four governorates (Sharkia, Ismailia, Beheira, and Dakahlia). The total biogenic amine content in Feseekh varied between 104.6 and 166.8 mg/kg. Six BAs Histamine (HIS), Tyramine (TYR), Putrescine (PUT), Cadaverine (CAD), Spermine (SPE), and Spermidine (SPD) were found in all fish samples under investigation. Histamine was quantitatively the most common biogenic amine in all samples, the histamine content ranged from 51.80 to 93.3 mg/kg, which was above the 50 mg/kg limits set by the Food and Drug Administration (FDA). Fermented fish (Feseekh) contains 14 amino acids. The essential amino acids accounted for 30.7-36.9% of the total concentration of amino acids. Fish flesh of mullet had the highest fatty acids that are saturated fatty was less than monounsaturated and polyunsaturated fatty acids. The total volatile basic nitrogen (TVBN) content was ranged from 23.00 to 48.00 mgN/100g, Thiobarbituric acid (TBA) content was from 1.36 to 2.94 mg malonaldehyde/kg sample and Trimethylamine (TMA) was from 3.20 to 23.10 mgN/100g. Meanwhile, The total bacteria count (TBC) was from (6.48 to 7.27 log CFU/g) was significantly higher than that of total mesophilic bacteria count (TMC) ranged from 6.04 to 7 log CFU/g. It could be inferred that the safety of Feseekh should be improved by hygienic manufacturing process.

Key words: Fish products, food analysis, food safety, biogenic amines, food microbiology.

INTRODUCTION

Fish are easily digestible, a great source of polyunsaturated fatty acids (PUFA), also a good source of minerals and vitamins (Kulawik et al., 2013). However, a highly destructive food that is spoiled shortly after death, unless properly preserved (Motaleb et al., 2010). Fish considered to have an excellent amino acid composition which ensures the recommendation of a strong and safe diet (Rabie et al., 2009).

Feseekh produced from one of Mugilidae family items salting to preserve them and to achieve an appropriate degree of aging, to provide them with their distinctive sensory properties. Dry or wet salt process is employed and products in sealed containers are stored Egyptian Organization for Standardization (EOS, 2005). Feseekh is a rich source of high-quality protein, essential amino acids, vitamins, and minerals (Rabie et al., 2009). Other amino acids, lysine, arginine, and tyrosine can potentially be transformed by microorganisms, present in the fermentation system into cadaverine, putrescine, and tyramine (Zaman et al., 2011).

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Salting has been historically one of the oldest techniques used in the preservation of fish in the world and is essentially intended to prevent spoilage and extending seafood shelf life. NaCl prevents microbial growth by limiting the water available in meat and fish products (i.e. decreasing water activity (aw)) (Aubourg and Ugliano, 2002). Furthermore, the salting process is still widely used around the world and a highly valued product, due to its high demand and its easy processing (Ghaly et al., 2010). In Egypt, the first type of feseekh has a low salt content, is suitable after 15-20 days of maturing, and the second has a high salt content and can be consumed after a 2-3 months (Rabie et al., 2009).

Biogenic amines are low molecular weight organic bases with biological activity that are produced in foods by microbial decarboxylation of the corresponding amino acids or by transamination by amino acid transaminases (Zhai et al., 2012). Hence storage temperature is an important factor contributing to the biogenic amine formation especially for fish that is exposed to warm waters or air which generate heat in their tissues (FDA, 2011). In fish products, the formation of biogenic amines is closely related to the activity of the microorganisms present in the fish. In the case of cured products, the massive quantity of particular biogenic amines may be produced as a result of poor first-class uncooked materials, microbial infection, adverse conditions in the course of processing storage, high temperatures, and excessive pH values (Xu et al., 2010). Also low salt concentrations can favor the accumulation of free amino acids, but can also stimulate the formation of biogenic amines. Therefore, it is important to estimate biogenic amines not from the point of view of the toxicity and freshness of the product, but also for the smooth conduct of the trade in seafood across continents (Biji et al., 2016).

Foods with a high level of biogenic amines (BAs) are considered unsafe as they can be related to various problems such as breathing irregularities, migraine, hypertension, and hypotension or allergies (Ladero et al., 2010). A total amount of 10 mg/100 g of biogenic amines in foodstuffs was considered harmful to human being and animal health (Shalaby, 1996). The risk action level for scombroid fish at the port has been set at 50 mg/kg (FDA, 2011). The European Food Safety Authority (Hazards, 2011), has recently set a maximum daily intake of histamine 50 mg and tyramine 600 mg for a healthy adult. Likewise, histamine should not exceed 200 mg/kg for salted, smoked, and frozen fishery products in Egypt (EOS, 2005). Total volatile nitrogen bases (TVB-N) are a general term that includes quantities of trimethylamine (TMA), dimethylamine (DMA), ammonia, and other volatile marine spoilage-related basic nitrogen compounds.

The goal of this study was to provide some additional information on biogenic amines and some chemical characteristics of traditional fermented fish (Feseekh), and their relationship to microbial load. Also, to state a fact related to the compatibly of mullet (Mugil cephalus) fish (Feseekh) with the Egyptian Organization for Standardization (EOS, 2005).

MATERIALS AND METHODS

Sampling

Fifteen samples of Feseekh were collected from different Egyptian local markets in four Governorates (Sharkia, Ismailia, Beheira, and Dakahlia), in June 2018. Each Feseekh sample weighted of approximately 1.5 to 2 kg was kept under cooling until analyses.

Physicochemical Composition

Moisture, crude protein, crude fat, and ash were determined according to the methods described in AOAC (2005). The pH value was determined according to the method mentioned by AOAC (2005), using digital pH meter (Type 3320 Jenway LTD, Felsted Dan mow Essex (M63 IB, UK). Total carbohydrate content was calculated by difference as follows: 100 - (% moisture + ash + protein + fat) (Mertens, 2005). These assays were conducted in the central lab, Faculty of Agriculture, Zagazig University, Egypt.

Thiobarbituric acid value (TBA) was measured according to the method described by Fernandez-Lopez et al. (2005). Total Volatile Basic-Nitrogen (TVB-N) was measured by steam-distillation of the TCA-fish extract, using the method of Malle and Tao (1987).
Trimethylamine (TMA) was carried out according to the modified method of \textit{Malle and Poumeyrol (1989)}. To block the primary and secondary amines, 20 mL formaldehyde was applied to the distillation flask. Steam distillation was then performed for the determination of TVB-N in TCA extract. When the required amount of formaldehyde was added, only the TMA was distilled. The TMA content was calculated from the volume of 0,025 N H$_2$SO$_4$ used for titration as follows:

\[ \text{TMA (mg N/100 g)} = 14 \text{mg/mol} \times a \times b \times 300/25 \text{ mL} \]

Where, \( a = \text{mL of sulphuric acid} \) \( b = \text{Normality of sulfuric acid} \). \( 14\) = the molecular weight of nitrogen.

**Colour Values**

The colour of mullet fish (Feseekh) was measured by using CIE colour values \( L^* \) (lightness), \( a^* \) (redness), \( b^* \) (yellowness) using Hunter colorimeter D65 illuminant (CM-400d, Konica Minolta, Tokyo, Japan), and 10° observer according to \textit{Singh et al. (2008)}. The chroma (\( C^* \)), hue angle (hub) and total colour difference (\( \Delta E \)) were calculated as \( C^* = 0.5, \text{hub} = \tan^{-1} (b^*/a^*) \) and \( \Delta E = ((L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2)^{1/2} \), where \( L_0, a_0 \) and \( b_0 \) are the lightness (\( L^* \)), red to green (\( a^* \)) and blue to yellow (\( b^* \)) values of the reference sample (\textit{Shih et al., 2003}).

**Determination of Amino Acids**

Amino acids composition of fish were carried out according to \textit{Black et al. (1958)} in the Central Lab. Faculty of Agriculture, Cairo University, Egypt. The amino acid analyzer type was 1-Sykon Automatic Amino Acid Analyzer (AAA400), INGOS Ltd, Germany. Acid hydrolysis was carried out according to the method of \textit{Black et al. (1958)}. The dried grounded sample (100 mg) was hydrolyzed with 6 N HCl (10 mL) in a sealed tube at 110°C in an oven for 24 h. The excess of HCl was then freed from 1mL hydrolyzed under vacuum of 80°C with occasionally addition of distilled water, then evaporated to dryness. The HCl free residue was dissolved in exact (2mL) of loading buffer (6.2M, pH = 2.2).

**Biogenic Amine Analysis**

Biogenic amines and free amino acids were extracted and determined in all tested samples according to \textit{Deabes et al. (2018)}. The analyses were carried out using High-Performance Liquid Chromatography (HPLC) (Hitachi, Tokyo, Japan), in the Department of Mycotic Toxins and Food Pollutants, National Research Centre, Egypt. For the extraction, twenty-five grams of homogenized Feseekh flesh were blended with 125 ml of 5% TCA (Trichloroacetic acid) for 3 min using a warning blender then filtration was achieved using filter paper Whatman No (1). Ten milliliters of extracts were transferred with 4g NaCl and 1 ml of 50% NaOH into a suitable culture tube, then shacked and extracted three times by 5 ml n-butanol; chloroform (1:1 v/v) stopped and shacked vigorously for 2 min. followed by centrifugation at 3000 rpm for 5 min. The upper layer was transferred using a disposable Pasteur pipette to a 50 ml separating funnel.15 ml of n-heptane was applied to the combined organic extracts (upper layer) and extracted three times with 1.0 ml portions of 0.2 N HCl and the HCl layer was collected in a glass stopper tube. With the help of a gentle air current, the solution was evaporated to dryness using a water bath at 95°C. The combined ether extracts were carefully evaporated with the aid of current air at 35°C in a dry bath. The obtained dry film was dissolved in 1ml methanol, then 10 µl were injected in HPLC.

**Fatty Acids Analysis**

Fatty acid methyl esters were prepared from total lipid by using a rapid method according to the method of \textit{ISO (2011)} by gas chromatography. Fatty acid methyl esters were prepared from total lipid by using a rapid method according to the method of \textit{ISO (2011)}. Fatty acid methyl esters were injected into HP 6890 series GC, (USA), apparatus provided with a DB-23 column (60 m × 0.25mm × 25 µm). The carrier gas was N$_2$ with a flow rate of 2.2 ml/min, splitting ratio of 1:50. The injector temperature was 250°C and that of Flame Ionization Detector (FID) was 300°C. The temperature setting was as follows: 150°C to 210°C at 5°C/min, and then held at 210°C for 25 min. Peaks were identified by comparing the retention times obtained with standard methyl esters.

**Microbiological Analyses**

For microbial determinations, twenty-five grams of each examined sample were aseptically weighed and transferred into a stomacher bag containing 225 ml sterile 0.1 % (w/v) buffered peptone water (BPW), and homogenized using a
Stomacher 400 Lab Blender (Seward Medical, London, UK) for 2 min to obtain the homogenate fluid of a dilution rate; (10^5). From the original homogenate, tenth fold serial dilutions up to 10^6 were then prepared according to Gilliland et al. (1984) method. 1-Total bacteria count: It was counted according to APHA (2001) using Plate Count Agar (Merck, 1.05463), where 0.1ml of each previously prepared serial dilution of the sample was spread into duplicate sterile Petri dishes, at selected dilutions. The plates were incubated at 37°C for 48 h. Colonies between “25-250” were counted and the total aerobic colony counts were then expressed as log cfu/g. 2-Total mesophilic bacteria were enumerated by using Plate Count Agar (PCA Merck, 1.05463), after incubation at 30 °C for 48 h, according to a method described in APHA (2001). 3- Lactobacillus was enumerated by using of de Man, Rogosa, Sharpe (MRS Biolife, Italy) overlain with the same medium (5 ml) and incubated for 72 h. at 25 °C according to the method of Frank et al. (1993). 4- Lactococcus sp. was enumerated by using M17 Agar (M17 Biolife, Italy), at 37°C for 72 h., based on method of Terzaghi and Sandine (1975). 5-Enterococci was detected by using Azide Dextrose agar (ADA, Biolife), after incubation at 28 °C for 48 h. according to Frank et al., (1993). 6-Salmonella and Shigella were detected by using Salmonella Shigella (SS) Agar (SSA Biolife), 37°C for 24 h., according to APHA (2001). 7- Staphylococcus was detected in Mannitol Salt Agar (MSA, Biolife), the plates were incubated at 37° C for 24 h., according to the methods recommended in Manual, (1982).

Statistical Analysis
The obtained data were statistically analyzed by a statistical for social science package SPSS version 20 program for windows, SPSS Inc according to Dominick and Derrick (2001).

RESULTS AND DISCUSSION
Physicochemical Composition of Feseekh Samples
Approximate analyses of the composition of Feseekh samples, moisture, crude protein, total lipids, ash, TVBN, TBA, TMA, and pH, are shown in Table 1. The moisture content of fish samples was found to be between 58.5 - 62%. During the later stage of processing, there has been a slight increase in the moisture content of salted fish as a result of absorption of salt by fish tissues as they swell when immersed into the salt solution for a long time (Wheaton and Lawson, 1985). This result agree with Mirsadeghi et al. (2019), who found that moisture contents of fish fillet of huso were significantly (p ≤ .05) ranging from 59.21 ± 0.33 to 78.99 ± 0.13 g/100 g. But similar result in moisture content of Climbing perch (Anabas testudineus) and Nile tilapia (Oreochromis niloticus), those caught from the pond was 64.03% and 69.83%. Whereas the moisture content of Anabas testudineus and Oreochromis niloticus) from open water was 65.02% and 70.26%, respectively (Mansur et al., 2019). The present results are lower than the fish species (anchovy, dogfish, and sardine) which showed that the average moisture content was around 77.95% (Silva et al., 2019).

The fat contents of fish samples were found to be between 5.42% - 7.36%. This finding was higher than that presented by Ahmed, (2006) who discovered that the fat content was (1.4 to 2.2%) and (1.62 to 0.88%) for fresh and salted fish, respectively. The crude fat content of the fish fillet of huso was statistically different ranging from 3.21 ± 0.08 to 5.4 ± 0.04 g/100 g (p ≤ .05) (Mirdadeghi et al., 2019).

The protein contents of collected Feseekh samples were found to be between 20.18 to 22.80 %. This result also agree with Bilgin and Gençcelep, (2015), who found that the protein content of canned solid tuna is between 20.61-22.87. The decrease of protein level was found to be significant, proportional to the salting treatment, as this is due to the protein being dissolved in the brine (Ojewola and Annah, 2006). The crude protein and ash ranged from 17.3 and 1.3% of cooled to 24.5 and 5.9% of salted sardine fish samples, respectively (Farag, 2013).

The pH level can induce amino acid accumulation and encourage the formation of biogenic amines (Hu et al., 2007). As shown in Table 1 pH of all examined samples were high, so samples may contain a low amount of biogenic
Table 1. Physicochemical composition of Feseekh samples collected from different Governorates in Egypt

| Governorate          | Moisture % | Fat % | Protein % | pH  | TVBN mg/kg | TBA mg/kg | TMA mg/kg | Ash % | Carbohydrate % |
|----------------------|------------|-------|-----------|-----|------------|-----------|-----------|-------|----------------|
| Sharkia (Abu Hammad)| 61.0 ab    | 7.24  ab| 22.19 ab  | 6.7 ab| 48.0 a     | 2.80 a    | 18.60 b   | 6.67 d| 2.89 b         |
| Sharkia (Zagazig)   | 62.0 a     | 7.36  a| 20.18 d   | 6.3 c| 23.80 d    | 1.36 c    | 7.60 c    | 4.79 a|                |
| Beheira              | 58.5 d     | 5.42  c| 21.20 c   | 6.5 bc| 32.00 c    | 1.80 bc   | 5.30 d    | 8.14 b| 5.47 a         |
| Ismailia             | 59.0 cd    | 6.68  b| 21.80 bc  | 6.6 b| 37.80 b    | 2.00 b    | 8.40 c    | 8.91 a| 3.30 b         |
| Dakahlia             | 60.0 bc    | 6.99  ab| 22.80 a   | 6.9 a| 45.60 a    | 2.94 a    | 23.10 a   | 7.34 c| 2.49 b         |
| F.test               | **         | **    | **        | **   | **         | **        | **        | **   | **            |
| L.S.D                | 1.396      | 0.654 | 0.709     | 0.250| 4.513       | 0.605     | 1.142      | 0.368 | 0.970         |

*Where ** highly significant at < 0.01. *a – d value with the same letter are not significantly different (P˃0.01). *a – d Means with a different letter are statistically significant P˂0.01). Thiobarbituric acid (TBA). Total Volatile Basic-Nitrogen (TVB-N). Trimethylamine (TMA).

Amines, pH value content of collected Feseekh samples were found between 6.3 - 6.9. This result is similar to that reported by Riebroy et al., (2008), who found that the pH in Thai-fermented fish mince for fresh fish is 6.3. On the other hand, this result is higher than that reported by Bilgin and Gençcelep, (2015), who found that the pH in canned fish samples is 5.22. Increases in pH indicate the accumulation of alkaline compounds, like ammonia and trimethylamine, derived mainly from microbial flora that is favourable for the increased activity of amino acid decarboxylase (Pons-Sánchez-Cascado et al., 2003).

Oxidative rancidity that is measured with thiobarbituric (TBA) test helps ensure that these salted fish have good shelf life. The TBA values were less than the maximum allowable level of all examined samples. TBA content was between 1.36 and 2.94 mg malonaldehyde/kg identified. Good quality fish and fishery products will have a TBA value of less than 2 while poor quality fish will have a TBA value between 3 and 27 (Bonnell and Thota, 1994). The decrease in TBA has been attributed to the interaction of decomposition products of protein with malonaldehyde to give tertiary products (Reddy and Setty, 1996). Also, this result is lower than the TBA values repored by Bilgin and Gençcelep, (2015) which were between 0.22-5.12 mg of MA/kg in fish products.

The TVB-N quality assessment is one way of determining the freshness of fish and fish products. The content of TVB-N of collected Feseekh was found between 23.80 to 48 mg/100 g. TVB-N values in canned fish samples TVB-N ranged from 5.57 to 47.23 mg/100 (Bilgin and Gençcelep, 2015). TVB-N values were lower than those obtained by Xu et al., (2009), who found that the content of TVB-N increased to 56.44 mg/100 g at 20 ± 1 C after 12 h of storage in Chinese mitten crab. TVB-N result was higher than obtained by (Arulkumar et al., 2016), who found that the level of TVB-N contents were 8.16 ± 0.80 mg/100 g, at 4°C and 33.86 ± 1.46 mg/100 g at 20°C after 24 h and TVB-N gradually increased in the blue swimmer crab after 96 hrs storage at 4°C and 20°C respectively.
The content of trimethylamine (TMA) is often used as a biochemical index to evaluate the holding efficiency and shelf-life of fish (Connell, 1990). Table 1 reveals that TMA was found in Feseekh samples between 3.20 – 23.10 mg/100 g. Due to the variable acceptance level, the TMA values vary according to species, season, storage conditions, bacteria, and activity of the intrinsic enzyme (Debvere and Boskou, 1996). This result is lower than that reported by Chouliara et al., (2005), who found that the TMA levels increased from less than 1 mg per 100 g of muscle to approximately 10 mg per 100 g of muscle after 42 days in non-irradiated sea bream fillets and 6–8 mg per 100 g of muscle in irradiated samples doses of 1 and 3 kGy. This result is also lower than that reported by Farag, (2013), who reported that frozen sardine fish products stored at 0°C for 8 weeks. TMA values decreased from 3.84 at zero time to 3.69 after 2 weeks but increased to 4.78 mg/100g after 8 weeks. Also TMA increases with increasing the storage period ranged from 3.84 at zero time to 32.5 mg/100g, after 4 months in salted sardine.

It is evident from the results achieved in Table 1 that the ash content of samples ranged from 6.67 to 8.91 %. The highest ash content was observed in the sample collected from Ismailia and Beheira (8.91 and 8.14%) respectively. While, the sample collected from Sharkia (Abu Hammad), contained the lowest level (6.67%). These results are higher regard to the permissible limits in the recommendation of EOS, (2005), that stated ash level should not be exceeded 3.00%. Furthermore, this result is higher than that obtained by Özden, (2005), who recorded ash level (1.92 and 1.76%) in marinated anchovy and trout were higher than those in fresh fish, the relative of ash and other components increased as a result of the water loss caused by the penetration of salt into the meat. A similar proximate composition (1.54% ash) has been reported for marinated fish (Arik et al., 2001).

**Colour Value**

The colour of fish products is a key parameter for the evaluation of some physical and chemical changes to the product can be assessed. Furthermore, it also has an important impact on sensory assessment. The data illustrated in Table 2 revealed that the maximum lightness (L*) value was 51.47 for the sample collected from Abu Hammad. Mean while, the minimum value was 28.47 for a sample collected from Ismailia. The maximum value of redness (a*) 5.55 was recworded for a sample collected from Beheira while, the lowest value 1.53 was for the samples collected from Ismailia. The maximum yellowness (b*) values was 9.37 for the sample collected from Abu-Hammad meanwhile, the minimum values were 6.34 for the samples collected from Ismailia. For whole products like Feseekh, a silvery appearance near the fresh product is considered to be of high quality. These results are higher than those reported by Rabie et al. (2018) during the storage period, starting with L * of 43.3 (control) and reaching 50 and 57.2 for Mackerel fish sauce treated with bromelain at 0.2 and 0.4 %, respectively, and fermented for 60 days. When the storage period was extended to 90 days, the L* values increased, reaching 58.6 and 64.1 for the samples with 0.2 and 0.4 % bromelain enzyme respectively. A study demonstrated that during the 270-day fermentation, the L* values of salt fermented fish sauce from anchovy was decreased while, and a* and b* values increased (Mueda, 2015). A decrease in a* value by 2.4 of salt fermented fish sauce from anchovy after 210 days but increased up to 4.9 after 270 days of fermentation was reported by Mueda (2015). Fish sauce treated with 0.2 and 0.4 % after 60 and 90 days of storage, respectively. The b* value of anchovy-fermented salt fish sauce increased from 4.2-66.1 after 270 days of fermentation (Mueda, 2015). The same researcher also reported that the initial tests of the values a* and b* showed that the colour properties of the samples were different from 28 days to 210 days and the samples of fish sauce were different from 270 days. Hue angle and chroma are the parameters associated with a* and b* values. In Table 2 the hue angle (h) of collected samples fish ranged from 54.60 to 76.60. The findings are lower than Rabie et al. (2018), who reported that during the fermentation period 77.7 to 83.6 for 90 days. Chroma value was ranged from 6.52 to 10.50 in the collected fish samples. This result is lower than that obtained by Rabie et al. (2018) ranged found 17.4 to 28.8 throughout the fermentation.
Table 2. Colour value of Feseekh sample collected from different Governorates in Egypt

| Governorate     | Colour value of Feseekh samples | L* | a   | b*  | c*  | h*  | ΔE |
|-----------------|---------------------------------|----|-----|-----|-----|-----|----|
| Sharkia (Abu Hammad) |                                | 51.47 a | 4.82 c | 9.37 a | 10.50" | 62.80 b | 33.00" |
| Sharkia (Zagazig) |                                | 49.71 b | 1.86 d | 7.86 b | 9.62 b | 54.80 c | 27.80 c |
| Beheira         |                                | 31.80 d | 5.55 a | 7.86 b | 7.86 b | 54.80 c | 27.50 c |
| Ismailia        |                                | 28.47 e | 1.53 e | 6.34 d | 6.52 d | 76.40 a | 30.80 c |
| Dakahlia        |                                | 36.76 e | 5.33 b | 7.49 c | 9.19 b | 54.60 c | 27.50 c |
| F. test         |                                | **    | **   | **   | NS   | **   | NS |
| L.S.D           |                                | 0.291 | 0.018 | 0.090 | 0.742 | 5.24  | 1.159 |

*Where ** refers to high significance at < 0.01. *a – d value with the same letter are not significantly different (P>0.01). *a – d Means with a different letter are statistically significant P<0.01). NS (Not significant). Colour values {L (lightness) “a (redness)” b (yellowness)}.

The non-essential amino acids in Feseekh samples included Glutamic acid (5.48–8.04 mg/100g), Alanine (3.96–5.0 mg/100g), Aspartic acid (5.13–8.45 mg/100g), Seronine (1.05-1.98 mg/100g), Proline (0.02-0.14 mg/100g), Glycine (4.27-6.11 mg/100g), Tyrosine (2.03-3.69 mg/100g), as shown in Table 3. These amino acids represented 63.0-69.2% of the total concentration of amino acids. Rabie et al. (2009), had obtained almost similar results where they found that after 60 days of fermentation of fish at room temperature to produce Feseekh, aspartic acid, glutamic acid, alanine, leucine, isoleucine, valine, and lysine were the main amino acids. Other amino acids of importance are lysine, arginine, and tyrosine as these can be potentially converted into cadaverine, putrescine, and tyramine, respectively, by microorganisms present in the fermentation system (Zaman et al., 2011). The main amino acids of salted fermented shrimp after 3 months were aspartic acid, glutamic acid, alanine, leucine, and lysine (Peralta et al., 2005). Besides, taurine can be considered as a major amino acid in philippine salted-fermented shrimp. The major precursors for biogenic amines are among the amino acids, lysine, tyrosine, and histidine. The glycine, lysine, and alanine may also be regarded as major contributors to the taste of seafood, and hence, they may contribute to the taste of Ikan Pekasam (Je et al., 2005). Free amino acids stored time for as long as 90 days. Also, ΔE value of collected sample fish ranged from 27.50 to 33.50. This result is lower than that obtained by Rabie et al., (2018) ranged found 64.4 to 49.1 throughout the fermentation time for as long as 90 days.

Amino Acids

A variety of amino acids such as glutamic acid, aspartic acid, alanine, and glycine are responsible for flavour and taste. Amino acids were also used as the quality indices for different species of fish and crustaceans (Ruiz-Capillas and Moral, 2004). Table 3 shows that 14 amino acids were detected in the collected feseekh samples.

The essential amino acids found in Feseekh were Leucine (0.92–1.51 mg/100g), Lysine (2.19–3.37 mg/100g), Valine (1.33–2.04 mg/100g), Isoleucine (0.74–1.11 mg/100g), Phenylalanine (0.67-1.19 mg/100g), Threonine (0.97–1.33 mg/100g), and Histidine (3.54-5.10 mg/100g) as shown in Table 3. These amino acids represented 30.7-36.9 % of the total concentration of amino acids. This result is lower than that repored by Jo et al. (2003), Rabie et al. (2009), reported that these amino acids represented 68 % of the total concentration of amino acids after 60 days of Feseekh storage.
Table 3. Amino acids concentration of Feseekh samples from different Governorates in Egypt

| Governorate             | Aspartic (mg/kg) | Glutamic (mg/kg) | Glysine (mg/kg) | Histidine (mg/kg) | Alanine (mg/kg) | Tyrosine (mg/kg) | Lysine (mg/kg) | Serine (mg/kg) | Valine (mg/kg) | Threonine (mg/kg) | Leucine (mg/kg) | Phenylalanine (mg/kg) | IsoLeucine (mg/kg) | Proline (mg/kg) | Total (mg/kg) |
|-------------------------|------------------|------------------|-----------------|-------------------|-----------------|------------------|----------------|---------------|---------------|---------------------|-----------------|----------------------|---------------------|----------------|---------------|
| Sharkia (Abu Hammd)    | 8.45a            | 8.04a            | 6.11a           | 5.10a             | 3.97b           | 3.69a            | 3.30a          | 1.98a         | 1.40b         | 1.33a               | 1.18ab          | 1.17ab                | 0.96ab             | 0.02b         | 46.70         |
| Sharkia (Zagazig)      | 6.80b            | 6.40b            | 6.00a           | 4.40b             | 5.00a           | 2.50c            | 2.50b          | 1.30b         | 1.60ab        | 1.00a               | 1.10b           | 1.10ab                | 0.80bc             | 0.14a         | 40.64         |
| Beheira                 | 6.88b            | 6.30b            | 5.19b           | 4.61b             | 3.96b           | 2.03c            | 2.55b          | 1.50ab        | 1.33b         | 1.16a               | 0.92b           | 0.81ab                | 0.74c              | 0.06b         | 38.04         |
| Ismailia                | 5.37c            | 5.61c            | 4.27c           | 3.54d             | 4.20b           | 2.83bc           | 2.19b          | 1.26b         | 1.36b         | 0.97a               | 1.01b           | 0.67b                | 0.74c              | 0.04b         | 34.06         |
| Dakahlia                | 5.13c            | 5.48c            | 4.38c           | 4.04c             | 4.86a           | 3.40ab           | 3.37a          | 1.05b         | 2.04a         | 0.98a               | 1.51a           | 1.19a                | 1.11a              | 0.05b         | 38.59         |
| F. test                 | **               | **               | **              | **                | *               | *                | **             | *             | *             | N.S                 | N.S             | N.S                  | *                   | *             |               |
| L.S.D                   | 0.918            | 0.377            | 0.347           | 0.331             | 0.526           | 0.807            | 0.457          | 0.527         | 0.500         | 0.498               | 0.352           | 0.517                | 0.218              | 0.068         |               |

*Where *, ** and NS refers to significance at 0.05, 0.01, and not significant, respectively. *a – d value with the same letter are not significantly different (P>0.01). *a – d Means with a different letter are statistically significant P<0.01
in Indian mackerel showed fluctuation in histidine concentration, significantly decreased after 12 h from 42.02 mg g\(^{-1}\) DW to 32.59 mg g\(^{-1}\) DW, then drastically decreased at the end of storage to a minimum value of 19.96 mg g\(^{-1}\) DW (Chong et al., 2014).

### Biogenic Amines

There have been individual detections of six biogenic amines (histamine, cadaverine, putrescine, tyramine, spermine, and spermidine) in the collected salted fermented fish Feseekh samples as shown in Table 4. Total biogenic amine levels of collected Feseekh samples ranged between 104.6 and 166.8 mg/kg. These findings are lower than that recorded by Zhai et al., (2012), who examined 49 fish products from the China market. It was observed that the average total BAs content of lightly cured horse mackerel was 484.42 mg/kg compared to 167.86 mg/kg or less for the other salted and fermented fish products. Furthermore, the amount of BA in control cheeses was reported to increase when the storage period increased (Rabie et al., 2011). Also, these findings are lower than that recorded by Zhang et al. (2011), who found that the total biogenic amine content in Layū (Layū is the traditional name for the dry-cured grass carp (Ctenopharyngodon idellus) produced in China), at 20°C was 773.4 mg/kg DW, above that stored at 4°C, 593.8 mg/kg DW. The maximum amount of 750–900 mg/kg total biogenic amines in foods was reported by Latorre-Moratalla et al. (2010). Therefore, overall biogenic amines from the different feseekh samples were below risk levels.

Histamine levels were above 50 mg/kg in sample of Sharkia (Abu Hammad), followed by Beheira and Ismailia (93.3, 64.80 and 91 mg/kg), respectively. The recorded values exceeded (EFSA, 2011), maximum allowed limit, recommending that histamine be allowed to maximally 50 mg/kg. The content of histamine (91 mg/kg) in salted fish brought from Ismailia was significantly higher (P<0.05) than 50 mg/100g, the allowable histamine limit suggested by FDA for scombroid fish/or product (EFSA, 2011; FDA, 2011). The EU has proposed that the average level of histamine in fish should not exceed 10 mg histamine per 100 g fish muscle, and it seems to be good for general health (Lehane and Olley, 2000). This result is lower than that obtained by Rabie et al. (2009), who has found histamine values of 21.1 mg/100 g in salted fermented fish Feseekh, after 60 days of storage, posing a health risk to the general public's use. Also, this result is similar to that obtained by Vosikis et al. (2008), who noted that histamine content ranged from 2.6 to 113.3 mg/kg in the anchovies. The histamine level by contrast was found to be 48.13 mg/100 g and 43.84 mg/100 g for milkfish and Indian whiting, respectively, after 24 h storage (Arulkumar et al., 2016). Also, histamine with an overall mean of 2662 mg/kg, was found to be the main biogenic amine in the Iranian fish sauce (Zarei et al., 2011).

Tyramine is a trace amine and a naturally occurring monoamine compound derived from the amino acid tyrosine. As represented in Table 4 tyramine concentrations in the samples collected from Beheira and Dakahlia were 22.86 and 17.10 mg/kg, respectively. This result is lower than that obtained by Xu et al. (2009), the high tyramine content ranged from 101 to 222 mg/kg in mackerel, Sauria, Spanish mackerel, and amberjack during ice storage. Comparable, 4.9 mg/kg tyramine was observed in blue scad during ice storage while, levels of 14.37 mg/100 g tyramine have recorded from orange-spotted grouper after 18 days storage in ice at 0°C (Bita et al., 2015). On contrary, tyramine concentrations were twice that of fresh water carp recorded by Křížek et al., (2004).

Putrescine is mainly produced by bacterial decarboxylation of ornithine (Özogul et al., 2006). The concentrations of putrescine in the examined samples of Feseekh were between 1.47 and 5.3 mg/kg as shown in Table 4. This result is lower than that obtained by Rabie et al., (2009), who established putrescine to be the second predominant amine in Egyptian salted-fermented fish samples, increasing from an initial level of 15 mg/kg (DW) to a 5-fold value during 20 days of ripening. The increase was further noted after 40 and 60 days of storage even more pronounced, 13 and 14 fold, respectively. In this respect Özogul et al., (2006), found putrescine values of less than 10 mg/kg have been proposed for carp meat of reasonable quality, values between 10 and 20 mg/kg for acceptable quality, and values over 20 mg/kg for carp meat of poor quality based on sensory scores.
Table 4. Biogenic amine concentration of Feseekh samples collected from different Governorates in Egypt (mg/kg).

| Governorate            | Biogenic amine concentrations of Feseekh sample | Histamine | Tyramine | Putrescine | Cadaverine | Spermine | Spermidine | Total  |
|------------------------|------------------------------------------------|-----------|----------|------------|------------|----------|------------|--------|
| Sharkia (Abu Hammad)   |                                                | 93.30a    | 5.22d    | 5.20a      | 3.70d      | 15.73c   | 32.69a     | 155.84 |
| Sharkia (Zagazig)      |                                                | 55.20d    | 8.00c    | 1.47c      | 2.90d      | 85.33a   | 1.70d      | 154.61 |
| Beheira                |                                                | 64.80c    | 22.86a   | 3.84ab     | 5.80c      | 65.50b   | 4.00c      | 166.8  |
| Ismailia               |                                                | 91.00b    | 5.70d    | 5.30a      | 7.50b      | 3.85e    | 1.60d      | 114.95 |
| Dakahlia               |                                                | 51.80e    | 17.10b   | 3.15b      | 11.05a     | 11.00d   | 10.50b     | 104.60 |
| F. test                |                                                | **        | **       | **         | **         | **       | **         | -      |
| L.S.D                  |                                                | 1.445     | 1.776    | 1.557      | 1.213      | 3.378    | 1.121      | -      |

* Where ** highly significant at < 0.01. *a – d value with the same letter are not significantly different (P>0.01). *a – d Means with a different letter are statistically significant P<0.01.

Cadaverine concentration is a good indicator of spoilage and is significantly associated with post-processing handling of fish products or post-harvest handling of fresh fish (Flic et al., 2001). Table 4 also showed that the concentration of cadaverine in the examined samples of Feseekh was within the range of 2.90 - 11.05 mg/kg. This result is less than what recorded after 20 days of Korean salted and fermented fish products Mah et al., (2002), the concentration of cadaverine increased from 480 to 1083–1205 mg/kg. This result was less than what was obtained by Rabie et al., (2009), cadaverine content recorded in Egyptian salted-fermented fish (Feseekh) was 997 mg/kg DW after 60 days of storage.

In most fish samples small amounts of spermidine were found, mainly because this amine plays an important role in live fish metabolism and cellular growth (Silla Santos, 1996). Concerning the concentration of spermidine in the examined samples, Feseekh from Abu Hammad and Dakahlia was the highest (32.69 and 10.5 mg/kg respectively), compared to other samples. But spermine concentration in Feseekh collected from Beheira and Zagazig recorded 65.5 and 85.33 mg/kg, respectively. This result is higher than that found by Rabie et al., (2009), which found that spermine (2 to 10 mg/kg DW). In another study, low contents of spermidine and spermine were recorded in smoked fish were 1.28 and 2.23 mg/100 g, 0.805 and 0.644 mg/100 g in sardine, and 0. 366 and 0. 871 mg/100 g in anchovy, after 120 days of storage, respectively (Rabie et al., 2014).

Fatty Acids

Fish are the best source of fatty acids (FA) particularly Poly Unsaturated Fatty Acids (PUFA). Fatty acid profile includes SFA (Saturated Fatty Acids), MUFA (Mono Unsaturated Fatty Acids) and PUFA. Data illustrated in Table 5 showed that the amount of unsaturated fatty acids (UFA) was found in the range of 47.77 to 53.68 mg/100g. The contents of total saturated fatty acids (SFA) have been detected from 38.34 to 46.02 mg/100 g. Mostafa and Salem (2015) found the highest fatty acid content in the SFA for mullet fish was palmitic acid, the palmitic acid contained in fresh fish flesh was 18.57%, after distension 31.03 and after 15, 30, 45 and 60 days were 21.24, 21.29, 22.58 and 24.12%, respectively. This result is higher than that reported by Rabie et al., (2018) the concentrations of palmitic acid were 1.4, 1.6, 1.7, 1.5, 1.8 and 1.9 mg/100 g in 60 and 90 days of fermentation for mackerel fish sauce, respectively.

The total concentrations of MUSFA were found between 20.01 to 23.86 mg/100g. In the MUFA, the highest fatty acid content for mullet
| Fatty acids                        | Sharkia (Abu Hammad) | Sharkia (Zagazig) | Beheira (Rashid) | Ismailia (altalalkair) | Dakahlia (MittGham) | F. test | L.S.D* |
|-----------------------------------|----------------------|-------------------|------------------|------------------------|---------------------|---------|--------|
| Myristic                          | 4.20 b               | 4.29 d            | 5.12 a           | 5.0 b                  | 4.85 c              | **      | 0.069  |
| Pentadecanoic                     | 2.02 b               | 1.49 c            | 2.12 a           | 1.95 c                 | 1.87 d              | **      | 0.0014 |
| Palmitic                          | 27.32 a              | 21.82 c           | 25.5 b           | 24.51 c                | 22.36 d             | **      | 0.223  |
| Margaric                          | 3.10 c               | 2.35 d            | 2.92 b           | 2.70 c                 | 2.75 c              | **      | 0.069  |
| Stearic                           | 3.60 b               | 3.18 c            | 3.72 a           | 3.07 d                 | 2.74 e              | **      | 0.041  |
| Arachidic                         | 0.65 a               | 0.35 d            | 0.59 b           | 0.48 c                 | 0.32 e              | **      | 0.007  |
| Behenic                           | 3.32 c               | 3.68 b            | 3.75 a           | 3.14 d                 | 2.91 e              | **      | 0.0015 |
| Lignoceric                        | 1.81 a               | 1.18 e            | 1.48 b           | 1.38 c                 | 1.22 d              | **      | 0.0012 |
| Total SFA                         | 46.02                | 38.34             | 45.20            | 42.23                  | 39.02               |         |        |
| Tetradecanoic                     | 0.70 c               | 0.83 b            | 0.63 d           | 0.92 a                 | 0.57 e              | **      | 0.0011 |
| Hexadecadienoic                   | 7.0 e                | 8.32 c            | 9.26 a           | 9.01 b                 | 7.16 d              | **      | 0.0017 |
| Haptadecenoic                     | 1.71 b               | 1.60 c            | 1.48 e           | 1.90 a                 | 1.54 d              | **      | 0.0048 |
| Oleic                             | 10.20 d              | 11.60 b           | 10.71 c          | 9.28 e                 | 13.15 a             | **      | 0.229  |
| Eicosenoic                        | 0.49 d               | 0.51 c            | 0.70 a           | 0.68 b                 | 0.45 e              | **      | 0.001  |
| Total MUFA                        | 20.1                 | 23.86             | 22.78            | 21.79                  | 22.87               |         |        |
| Hexadecadienoic                   | 2.40 e               | 3.05 a            | 1.88 c           | 2.20 d                 | 2.66 b              | **      | 0.0021 |
| Linoleic                          | 5.20 c               | 5.04 d            | 6.69 a           | 6.51 b                 | 6.73 a              | **      | 0.046  |
| g- Linolenic                      | 0.60 b               | 0.26 c            | 0.33 d           | 0.39 c                 | 1.40 a              | **      | 0.0084 |
| a- Linolenic                      | 8.11 c               | 7.20 c            | 7.7 d            | 9.90 a                 | 8.95 b              | **      | 0.0215 |
| Octadecatetraenoic                | 2.20 b               | 2.33 a            | 1.48 d           | 2.20 b                 | 1.76 c              | **      | 0.010  |
| Archidonic                        | 4.96 b               | 5.55 a            | 5.11 b           | 4.76 c                 | 4.71 c              | **      | 0.198  |
| Ardenic                           | 4.20 c               | 6.21 a            | 3.92 d           | 3.30 c                 | 4.60 b              | **      | 0.143  |
| Total PUFA                        | 27.67                | 29.64             | 27.11            | 29.26                  | 30.81               |         |        |
| Total UFA                         | 47.77                | 53.50             | 49.89            | 51.05                  | 53.68               |         |        |

*Where ** refers to high significance at < 0.01. * *a – d value with the same letter are not significantly different (P>0.01). *a – d Means with a different letter are statistically significant P<0.01. 
fish was Oleic acid, which was the highest contained in fish flesh after distension 14.0%. Mostafa and Salem (2015), found in the MUFA, the highest fatty acid content for mullet fish was C18:1 (Oleic acid), which was the highest contained in fish flesh after distension 21.22%.

The total concentrations of PUFA were found between 27.11 to 30.81 mg/100g. The highest content of fatty acid in PUFA was (Linolenic acid), which is the highest contained in fish of 10.61%. Mostafa and Salem (2015), found the highest content of fatty acid in PUFA was C18:2 (Linoleic acid), which is the highest oleic acid contained in fresh fish flesh of 10.49%. The contents of polyenes in the muscle of fresh anchovy and rainbow trout were 36.23 and 33.88%, respectively, somewhat lower than in marinated fish (anchovy, 34.14%; trout, 33.27%) (Ozdan, 2005).

**Microbiological Estimation of Feseekh**

Microbiology estimations of bacterial numbers are required to quantitatively associate with foodstuff characteristics during storage to serve the purpose of food safety and shelf-life determination (Dalgaard, 2000). Table 6 displays the mean values of the Total bacteria count (TBC) of fish flesh from mullet fish (Feseekh) were between 6.48 - 7.27 log CFU/g. This result is lower than that observed by Eltom (1989), who stated that after adding salt in fish fermentation, on the fourth day, the viable count increased to 1.8 x 10⁸ CFU/g, then gradually decreased to 8.6 x 10⁵ CFU/g on the twelfth day. In line with this results, TPC increased to CFU/g 6.7 and 8.0 log after one and two days of mackerel stored respectively at 25°C (Jiang et al., 2013). The total bacterial count of Feseekh should not exceed 10⁶ CFU/g. The coliform group should be less than 10³ and 10⁴ CFU/g in frozen and smoked fish, respectively (EOS, 2005). But this result is lower than those stated by Jiang et al. (2013), finding that TPC increased on day one to 6.7 log CFU/g and on day two stored mackerel was 8.0 log CFU/g at 25°C. The present results are in agreement with Jeya Shakkla et al. (2002), who recorded that the total bacterial load in the salted fish ranged from 10⁴ to 10⁵ CFU/g. Furthermore, Jeya Shakkla et al. (2002), stated that maximum bacterial load in samples of cured (salted, dried, and smoked) fishery products ranged from 10⁵ to 10⁶ CFU/g.

Table 6 shows the mean values of Total Mesophilic Bacteria (TMB) were in the range of 6.04-7.0 log CFU/g. In this context, Arulkumar et al. (2016), concluded that the total mesophilic bacterial growth in milkfish and Indian whiting fish increased after 18 h of ambient temperature storage. The total mesophilic bacterial in the present study was exceeded the maximum acceptable level of 6.04–7.0 log CFU/g for collected Feseekh samples. While, this result is higher than those found by Patir et al. (2006), who reported that the total mesophilic aerobic bacterial counts in salted grey mullet (Chalealburnus tarichii) stored at 4±1°C ranged from 2.0 to 5.0 log CFU/g, with an average of 3.94 log CFU/g.

**Enterococci** can affect the maturing process due to their proteolytic and lipolytic activity and their ability to stimulate the production of acid by certain lactococci (Sarantinopoulos et al., 2001). Table 6 shows the mean values of Enterococci of selected Feseekh were ranged from 3.05 to 4.62 log CFU/g. Tyramine-producing enterococci have been isolated from different sources, such as meat. Enterococci, which typically contaminate raw meat in the 10²–10⁴ CFU/g range and are highly resistant to extremes in temperature, pH, and salinity, can multiply to high numbers and act as spoiling agents in processed meat (Capozzi et al., 2011). Enterococci tyrosine decarboxylase (TDC) has also been shown to be capable of decarboxylating phenylalanine, an amino acid structurally similar to tyrosine, from which BA phenylethylamine originates cheese, fish and wine (Capozzi et al., 2011).

Table 6 shows the mean values of Staphylococcus were ranged from 3.04 to 4.68 log CFU/g. Staphylococcus should be less than 10⁵ in salted (EOS, 2005). Bhatia and Zahoor, (2007) found that 1 μg of Staphylococcal enterotoxins can be reached when the cell number exceeds 10⁵ CFU/g of food. As a preventive measure, legal limits of 10⁵ -10⁶ CFU/g had been set for Staphylococcus aureus in different fishery products.
Table 6. Microbiological estimation (log cfu/g) of Feseekh samples collected from different Governorates in Egypt

| Governorate       | Microbiological estimation (log cfu/g) of Feseekh |
|-------------------|-----------------------------------------------|
|                   | TBC   | TMB   | Lactobacilli | Lactococci | Enterococci | Staphylococci | Salmonella, Shigella |
| Sharkia (AbuHammad) | 7.16<sup>a</sup> | 7.00<sup>a</sup> | 5.48<sup>b</sup> | 4.49<sup>b</sup> | 4.30<sup>b</sup> | 4.40<sup>b</sup> | 2.55<sup>b</sup> |
| Sharkia (Zagazig)  | 6.48<sup>b</sup> | 6.04<sup>d</sup> | 4.14<sup>d</sup> | 3.30<sup>d</sup> | 3.05<sup>d</sup> | 3.04<sup>d</sup> | 1.95<sup>d</sup> |
| Beheira            | 7.20<sup>a</sup> | 6.67<sup>c</sup> | 4.75<sup>c</sup> | 4.11<sup>c</sup> | 3.23<sup>c</sup> | 3.26<sup>c</sup> | 2.41<sup>c</sup> |
| Ismailia           | 7.27<sup>a</sup> | 6.73<sup>c</sup> | 5.21<sup>b</sup> | 4.75<sup>a</sup> | 3.30<sup>c</sup> | 3.36<sup>c</sup> | 2.85<sup>a</sup> |
| Dakahlia           | 7.20<sup>a</sup> | 6.90<sup>b</sup> | 5.59<sup>a</sup> | 4.49<sup>b</sup> | 4.62<sup>a</sup> | 4.68<sup>a</sup> | 2.92<sup>a</sup> |
| **F. taet**        | **** | **** | **** | **** | **** | **** | **** |
| L.S.D             | 0.197 | 0.099 | 0.213 | 0.098 | 0.091 | 0.138 | 0.90 |

*Where ** highly significant at < 0.01. *a – d value with the same letter are not significantly different (P˃0.01). *a – d Means with a different letter are statistically significant P<0.01). Total bacteria count (TBC). Total mesophilic bacteria (TMB).

Table 6 shows that the mean values of Salmonella & Shigella were ranged from 1.95-2.92 log CFU/g. The bacterial Ornithine Decarboxylase (ODC), alignments showed two separated groups, one of them includes several proteins from Gram-positive bacteria, such as from some Lactobacillus strains, and Gram-negative bacteria (E. coli, Haemophilus influenzae, Salmonella typhimurium, Shigella flexneri, Vibrio cholerae, Vibrio parahaemoliticus, and Yersinia pestis strain) (De Las Rivas et al., 2006). Staphylococcus xylosusas, a halotolerant bacterium from dried flying fish products capable of producing more than 300 ppm of histamine at a concentration of 3% sodium chloride (Kung et al., 2015). Some of the LAB strains belonging to Lactobacillus, Lactococcus, Leuconostoc, Streptococcus, and Enterococcus genera can decarboxylate tyrosine and lysine (Omafuvbe and nyioha, 2011).

Table 6 reveals the mean values of Lactobacilli were ranged from 4.14 to 5.59 log CFU/g. Indicated that a significant proportion of LAB of the whey was represented by Lactobacillus sp. (5.36 ± 0.08 log CFU/ml) rendering it a viable inoculum for fermentation (Burns and Bagley, 1996). Also, it shows the mean values of Lactococcus ranged from 3.32 to 4.75 log CFU/g. Indicate that fermentation by whey (the watery part of milk that remains after the formation of curds) or L. acidophilus can substantially reduce the population of Staphylococcus, Clostridium and coliform bacteria, with the latter being totally removed from the WFFO (whey-fermented fish offal) and faecal coliform removed from the LAFFO (L. acidophilus-fermented fish offal) (Samaddar and Kaviraj, 2014).

**Conclusion**

Fermented fish (Feseekh) are a public food product in the Egyptian market; however, there are safety problems regarding this product owing to microbial contamination during processing that lead to biogenic amines formation. So, the present study was aimed to give some additional information about biogenic amines in traditionally fermented fish (Feseekh) and its relation to microbial load, determine the lipid, protein, moisture, TVBN, TBA, TMA , pH, and ash of salted fish and changes in amino acid and fatty acid composition to investigate the possibility of using these changes as quality indices. And to state a fact related to the compatibly of mullet (Mugil cephalus) fish (Feseekh) with the (EOS, 2005).

**REFERENCES**

Ahmed, I.O. (2006). Comparison of the nutritive value of Feseekh using Hydrocynus spp and Schilbe spp. Ph.D. Thesis, AlNeelain Univ., Khartoum, Sudan.

APHA (2001). (American Public Health Association): Compendium of Methods for Microbiological Examination of Foods, 4th...
AOAC (2005). Official Method of Analysis. Association of Official Analytical Chemists, 16th Ed., Gaithersburg, DC, USA

Arik, F., F. Fiedler, M. Lukowicz, B. Sperner and A. Stolle (2001). Untersuchungen zur Haltbarkeit von be- und verarbeiteten Süßwasserfischen. Archiv für Lebensmittelhygiene, 52: 34-39.

Arulkumar, A., K. Gunasekaran., P. Sadayan and A. R.Mohamed (2016). Histamine levels in Indian fish via enzymatic, TLC and HPLC methods during storage. J. Food Measur. and Characterization,11: 281–289.

Aubourg, S.P. and M. Ugliano (2002). Effect of brine pretreatment on lipid stability of frozen horse mackerel (Trachurus trachurus). Eur. Food Res. and Technol., 215: 91–95.

Bhatia, A. and S. Zahoor (2007). Staphylococcus aureus enterotoxins: A review. J. Clin. Diag. Res., 3: 188-197.

Biji. K.B., C.N. Ravishankar, R. Venkateswarlu, C.O. Mohan and T.K. Srinivasa Gopal (2016). Biogenic amines in seafood: A review. J. Food Sci. Technol., 53 (5): 2210–2218.

Bilgin, B. and H. Genccelep (2015). Determination of biogenic amines in fish products. Food Sci. and Biotechnol., 24: 1907-1913.

Deabes, M.M., K.H. Naguib, A.M. Ayesh, E.M. El-Damaty and G.H. Rowayshed (2018). Comparison of the HPLC and the TLC techniques for the determination of biogenic amines spiked to sausage and smoked herring samples. Enliven: Toxicol Allied Clin Pharmacol., 5 (1) : 001

De las rivas, B., A. Marcobal, A.V. Carrascosa and R. Munoz (2006). PCR detection of foodborne bacteria producing the biogenic amines histamine, tyramine, putrescine, and cadaverine. J. Food Prot., 69: 2509-2514.
Dominick, S. and R. Derrick (2001). Theory and problems of statistics and econometrics. 2nd Ed. New York, 202.

EFSA (2011). Panel on Biological Hazards (BIOHAZ): Scientific opinion on risk base control of biogenic amine formation in fermented foods. EFSA J., 9: 2393-2486.

Eltom, A. (1989). Microbiology and biochemistry of Fessekh fermentation. M. Thesis. Univ. Khartoum, Sudan.

EOS (2005). Egyptian Organization for Standardization. Salted Fish, Part: 1 Feseekh, Egyptian Organization for Standardization and Quality, Arab Republic of Egypt. No. 1725-1.

Farag, M. M.A. (2013). Estimation of formatting biogenic amines Concentration in fresh and processed sardine fish products during different storage conditions. World J. Fish and Marine Sci. 5 (6): 628-636.

FDA, U. (2011). Food and Drug Administration. CFR-Code of Federal Regulations Title, 21.

Fernandez, L.J., J. Zhi Aleson, A. Perez-Alvarez and V. Kuri (2005). Antioxidant and antibacterial activities of natural extracts: Application in beef meatballs. Meat Sci., 69: 371-380.

Flic, J.G., M.P. Oria and L. Douglas (2001). Potential hazardous in cold smoked fish: Biogenic amines. J. Food Sci., 66:1088-1099.

Frank J.F., G.L. Christen and L.B. Bullerman (1993). Tests for groups of microorganisms. In: Marshall, R (Ed.), Standard Methods for the Examination of Dairy Products, 16th Ed. Ame. Public Health Assoc., Washington DC, 271–286.

Ghaly, A.E., D. Dave, S. Budge and M.S. Brooks (2010). Fish spoilage mechanisms and preservation techniques: Rev. Am. J. Appl. Sci., 7: 846–864.

Gilliand, S., F. Bustø and J. Brinda (1984). Compendium of Methods for the Microbiological Examination of Foods, Ed. Speck, ML. Am. PubI. Health Assoc. Inc, Washington DC.

Hazards, E.P.O.B. (2011). Scientific opinion on risk based control of biogenic amine formation in fermented foods. Europ. Food Safety Authority (EFSA), J., 9: 2393.

Hu, Y., W. Xia and X. Liu (2007). Changes in biogenic amines in fermented silver carp sausages inoculated with mixed starter cultures. Food Chem., 104: 188-195.

ISO (2011). Animal and vegetable fats oils-Gas chromatography of fatty acid methyl esters. 12966-2, first edition.

Je, J.Y., P.J. Park, W.K. Jung and S.K. Kim (2005). Amino acid changes in fermented oyster (Crassostrea gigas) sauce with different fermentation periods. Food Chem., 91: 15–18.

Jeya Shakila, R., R. Lakshmanan and G. Jeyasekaran (2002). Incidence of amine forming bacteria in the commercial fish samples of Tuticorin region. Indian J. Microbiol., 42: 147-150.

Jiang, Q.Q., Z.Y. Dai, T. Zhou, J.J. Wu, J.Z. Bu and T.L. Zheng (2013). Hisamine production and bacterial growth in mackerel (p neumatophorus japonicus) during storage. J. Food Biochem., 37: 246-253.

Jo, C., D. Kim, M. Shin, I. Kang and M. Byun (2003). Irradiation effect on bulgogi sauce for making commercial Korean traditional meat product, bulgogi. Radiation Physics and Chem., 68: 851-856.

Křížek, M., F. Vácha, L. Vorlová, J. Lukášová and S. Cupáková (2004). Biogenic amines in vacuum-packed and nonvacuum-packed flesh of carp (Cyprinus carpio) stored at different temperatures. Food Chem., 88: 185-191.

Kulawik, P., F. Ozogul and H.G. Robert (2013). Quality properties, fatty acids, and biogenicamines profile of fresh tilapia stored in ice. J. Food Sci., 78 (7): S1063.

Kung, H.F., C.Y. Huang, C.M. Lin, L.H. Liaw, Y.C. Lee and Y.H. Tsai (2015). The histamine content of dried flying fish products in Taiwan and the isolation of halotolerant histamine-forming bacteria. J. Food and Drug Anal., 23: 335-342.
Ladero, V., M. Calles-Enriquez, M. Fernandez and M.A. Alvarez (2010). Toxicological effects of dietary biogenic amines. Curr. Nutr. Food Sci., 6: 145-156.

Lehane, L. and J. Olley (2000). Histamine fish poisoning revisited. Int. J. Food Microbiol., 58: 1-37.

Latorre-Moratalla, M.L., S. Bover-Cid, R. Talon, M. Garriga, E. Zanardi, A. Ianieri, M. J. Fraqueza, M. Elias, E. H. Drosinos and M.C. Vidal-Carovu (2010). Strategies to reduce biogenic amine accumulation in traditional sausage manufacturing. Eur. Food. Res. Technol., 43: 20-25.

Malle, P. and S.H. Tao (1987). Rapid quantitative determination of trimethylamine using steam distillation. J. Food Prot., 50: 756-760.

Malle, P. and M. Pounneyrol (1989). A new chemical criterion for the quality control of fish: Trimethylamine/Total Volatile Basic Nitrogen(%). J. Food Prot., 52: 419-423.

Mah, J.H., H.K. Han, Y.J. Oh, M.G. Kim and H.J. Hwang (2002). Biogenic amines in Jeotkals, Korean salted and fermented fish products. Food Chem., 79: 239–243

Manual, O. (1982). The oxoid manual of culture media ingredients and other laboratory services. Oxide limited, Basingstoke, Hampshire, England.

Mansur, M.A., M.N. Uddin, S. Akpar, M.N. Haider, M.D. Manik Miai, U.K. Salma and D.D. Kimura (2019). Comparative study on the quality and safety aspects of Climbing perch (Anabas testudineus) and Nile tilapia (Oreochromis niloticus) from pond and open water of Mymensingh, Bangladesh. Bangladesh J. Fish., 31(1): 119-124.

Mostafa, A. and R. Salem (2015). Characterization of microbiological and nutritional Variations in processed mullet (Mugil cephalus) Fish. Int. J. Microbiol. Res. 6 (2): 108-122.

Motalebi, A.A., A.A. Hasanzati, A.A. Khanipour and M. Soltani (2010). Impacts of whey protein edible coating on chemical and microbial factors of gutted Kilka during freeze storage. Iranian J. Fisheries Sci., 9 (2): 255-264.

Mertens, D. (2005). AOAC Official Method 975.03. In: Horwitz W, Latimer GW (eds) Metal in plant and pet foods. Official Methods of Analysis, 18th Ed., 3-4.

Mirsadeghi, H., A. Alishahi, M. Ojagh and P. Pourashouru (2019). The effect of different kinds of chitosans and cooking methods on the formation of heterocyclic aromatic amines in huso (Huso huso) fillet. J. Food Proc. Preserv., 43: 14253.

Mueda, R.T. (2015). Physico-chemical and color characteristics of salt fermented fish sauce from anchovy Stolephorus commersonii. Aquaculture, Aquarium, Conservation and Legislation, 8: 565-572.

Ojewola, G.S. and S.I. Annah (2006). Nutritive and economic value of danish fish meal, crayfish dust meal and shrimp waste meal inclusion in broiler diets. Int. J. Poul. Sci., 5: 390-394.

Omafuvbe, B.O. and E.L.C. Nyioha (2011). Phenotypic identification and technological properties of lactic acid bacteria isolated from selected commercial Nigerian bottled yoghurt. African J. Food Sci., 5: 340-348.

Özden, Ö. (2005). Changes in amino acid and fatty acid composition during shelf-life of marinated fish. J. Sci. Food and Agri., 85, 2015-2020.

Ozogul Y, F. Ozogul and C. Gokbulut (2006). Quality assessment of wild European eel (Anguilla anguilla) stored in ice. Food Chem., 95:458–465.

Patir, B., A.G. Inanli, G. Oksuztepe and O.I. Ilhak (2006). Microbiological and chemical qualities of salted Grey Mullet (Chalcalburnus tarichii) PALLAS, 1811). Int. J. Sci. and Technol., 1: 91-98.

Peralta, E.M., H. Hatate, D. Watanabe, D. Kawabe, H. Murata, V. Hama and R. Tanaka (2005). Antioxidative activity of philippine salt-fermented shrimp and variation of its constituents after fermentation. J. Oleo Sci., 54 (10): 553–558.

Pons-sanchez-Cascado, S., M.T. Veciana-Nogues and M.C. Vidal Carou (2003). Effect of delayed gutting on biogenic amine contents.
during ripening of European anchovies. Eur. Food. Res. Technol., 216: 489-493.

Rabie, M., L. Simon-Sarkadi, H. Siliha, S. Elseedy and A.A. El Badawy (2009). Changes in free amino acids and biogenic amines of Egyptian salted-fermented fish (Feseekh) during ripening and storage. Food Chem., 115: 635-638.

Rabie, M.A., S. El-Saidy, A.A. El-Badawy, H. Siliha and F.X. Malcata (2011). Biogenic amine contents in selected Egyptian fermented foods as determined by ion-exchange chromatography. J. Food Prot., 74: 681-685.

Rabie, M.A., A.O. Toliba, A.R. Sulieman and F.X. Malcata (2014). Changes in biogenic amine contents throughout storage of canned fish products. Pak. J. Food Sci., 24 (3):137–150.

Rabie, M.A., M. Namir, N.A. Rabie and M.F.R. Hassanien (2018). Acceleration of mackerel fish sauce fermentation via bromelain addition. Nutr. and Food Sci., 49: 47-61.

Reddy, K.P. and T.R. Setty (1996). An intermediate moisture product from mackerel (Rastrelliger kanagurta) using salt curing, fermentation, and drying. J. Aquatic Food Product Technol., 5: 65-82.

Riebroy, S., S. Benjakul and W. Visessanguan (2008). Properties and acceptability of Somp-fug, a Thai fermented fish mince, inoculated with lactic acid bacteria starters. LWT-Food Sci. and Technol., 41: 569-580.

Ruiz-Capillas, C. and A. Moral (2004). Free amino acids in muscle of norway lobster (Nephrops norvegicus (L.)) in controlled and modified atmospheres during chilled storage. Food Chem., 86:85–91.

Samaddar, A. and A. Kaviraj (2014). Processing of fish offal waste through fermentation utilizing whey as inoculum.Int. J. Recycl. Org. Waste Agricult., 3 : 45.

Sarantinopoulos, P., C. Andrighetto, M.D. Georgalaki, M.C. Rea, A. Lombardi, T.M., Cogan, G. Kalanzopoulos and E. Tsakalidou (2001). Biochemical properties of enterococci relevant to their technological performance. Int. Dairy J., 11: 621-647.

Shalaby, A.R. (1996). Significance of biogenic amines to food safety and human health. Food Res. Int., 29:675–690.

Shih, L., L.G. Chen, T.S. Yu, W.T. Chang and S.L. Wang (2003). Microbial reclamation of fish processing wastes for the production of fish sauce. Enzyme and Microbial Technol., 33: 154-162.

Silla Santos, M.H. (1996). Biogenic amines: their polyamines in food and their importance in foods. Int. J. Food Microbiol., 29 (2): 213-231.

Silva, A., A. Leonel, J. Veit, A. Feiden and R. Coutinho (2019). Semi preserved of marine fish, physical chemical, microbiological and nutritional characterization. Int. J. Advanced Eng. Res. and Sci. (IJAERS), 6: 10.

Singh, S., C.S. Riar and D.C. Saxena (2008). Effect of incorporating sweet potato flour to wheat flour on the quality characteristics of cookies. Afr. J. Food Sci., 2:65-72.

Terzaghi, B.E. and W. Sandine (1975). Improved medium for lactic streptococci and their bacteriophages. Appl. Environ. Microbiol., 29: 807-813.

Vosikis, V., A. Papageorgopoulou, V. Economou, S. Frillingos and C. Papadopoulou (2008). Survey of the histamine content in fish samples randomly selected from the Greek retail market. Food Additives and Contaminants, 1: 122-129.

Wheaton, F.W. and T. B. Lawson (1985). Other preservation methods. In: Processing Aquatic Food Products, pp: 273-328. John Wiley and Sons, New York.

Xu, Y., W. Xia and J.M. Kim (2009). Biogenic and volatile amines in Chinese mitten crab (Eriocheir sinensis) stored at different temperatures. Int. J. Food Sci. and Technol., 44: 1547-1552.

Xu, Y., W. Xia, F. Yang, J. M. Kim and X. Nie (2010). Effect of fermentation temperature on the microbial and physicochemical properties of silver carp sausages inoculated with Pediococcus pentosaceus. Food Chem., 118: 512–518.
Zaman, M.Z., F. Abu Bakar, S. Jinap and J. Bakar (2011). Novel starter cultures to inhibit biogenic amines accumulation during fish sauce fermentation. Int. J. Food Microbiol., 145: 84–91.

Zarei, M., H. Najafzadeh, M.H. Eskandari, M. Pashmforoush, A. Enayati, D. Gharibi and A. Fazlara (2011). Chemical and microbial properties of mahyaveh, a traditional Iranian fish sauce. Food Control, 23(2): 511-514.

Zhai, H., Y. Xianqing, L. Laihao, X. Guobin, C. Jianwei, H. Hui and H. Shuxian (2012). Biogenic amines in commercial fish and fish products sold in southern China. Food Control, 25: 303-308.

Zhang, J., Z. Liu, Y. Hu, Z. Fang, J. Chen, D. Wu and X. Ye (2011). Effect of sucrose on the generation of free amino acids and biogenic amines in Chinese traditional dry-cured fish during processing and storage. J. Food Sci. and Technol., 48: 69-75.