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Chemical profile of salted and smoked fish

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ABSTRACT

The current study was established to investigate the chemical profile of salted and smoked fish at retail markets in 90 meat products samples of smoked herring, salted sardine, and fesiekh (30 of each) were collected from two retail sales markets in Garbia,Governorate, Egypt. Ninety random selected samples of smoked herring, salted sardine, and fesiekh (thirteen (30) of each) were gathered from various markets at Gharbia governorate, Egypt, at different times. The collected samples were been submitted immediately to the laboratory for an expedited evaluation after being kept apart in an insulated ice box. The keeping quality tests (pH, TVB-N, and TMA and TBA), salt content and heavy metal residues (mercuryand lead) in examined samples which determined according to official methods. The average pH level was 6.18±0.03, 6.40±0.05, and 6.49±0.05 for the smoked herring, salted sardine, and fesiekh, respectively. Concerning Total volatile Basic Nitrogen(TVB-N) mean values were 19.56±1.17, 24.08±1.32, and 28.73±1.49 mg/100g, for smoked herring, salted sardine, and fesiekh, respectively, while Trimethylamine (TMA) values for the smoked herring, salted sardine, and fesiekh were 4.71 ±0.39, 6.98± 0.55 and 9.04± 0.67mg/100g, respectively. The average Thiobarbituric Acid Number (TBA) values in the smoked and salted fish products under investigation were 2.62± 0.14, 3.36±0.18, and 3.54±0.25 mg malondialdehyde/kg, respectively. Additionally, the analyzed smoked herring, salted sardine, and fesiekh had salt percentages was 6.33± 0.02%,6.87± 0.04, and 7.15± 0.05, respectively. Further, the analyzed smoked herring, salted sardine, and fesiekh had mercury residues of 0.72±0.01, 0.98±0.0, and 0.46±0.01mg/kg, respectively. Moreover, the lead residues were 0.25± 0.01, 0.41± 0.01, 0.46± 0.02, and mg/kg in the smoked herring, salted sardine, and fesiekh, respectively. There are safety concerns with these products when it comes to salting or smoking. One of the main problems is limiting the quality, acceptability, and shelf life of certain fish products. Fish products that have been smoked and slated are customarily consumed in Egypt on a variety of occasions. There are safety concerns with these products when they come to salting or smoking.

1. INTRODUCTION

Unsaturated fatty acids and fish are both recognized to have great amino acid compositions and to contribute to a balanced, nutritious diet. Due to its composition, fish is the most perishable food product, and the primary goal of the fish industry is to increase fish quality. One of the earliest ways of food preservation is salting, which preserves fish using dry edible salt (fesiekh and moluoha) or is similar to pickling (sardine). By osmosis, salt prevents the growth of microbes by removing water from their cells.

Most undesirable bacterial species must be killed with salt concentrations of up to 20%. Fish that has been salted is frequently prepared by smoking (smoked salted herring). Because salt is hypertonic and most bacteria, fungi, and other potentially pathogenic organisms cannot thrive in it, salting is employed in food preparation (FAO2005). Microorganisms and their autolytic enzymes break down the protein, carbohydrate, and lipid components of tissue to produce simpler metabolites including lactic acid, dimethylamine (DMA), and Thiobarbituric acid (TBA) which can be used as a gauge of fish quality since the concentration of these metabolites in tissues causes variations in their sensory characteristics and chemical composition (FAO, 1980).

lead, cadmium, and mercury are harmful to humans also at low amounts when consumed over an extended period, exposure to these heavy metals poses the greatest hazards to human health. The goal of the current study is to *investigate the* keeping quality tests (pH, TVB-N, and TMA and TBA), salt content, and heavy metal residues (mercury and lead) in examined samples. According to the FAO (2005), heavy metal can be collected in their tissues to levels several hundred times greater than those found in the surrounding water.

Chemical changes are detected by chemical analysis, to identify degradation level and compounds formation and infer the quality of the fish (Ana et al., 2020). pH provides indications of physical changes occurring in fish muscle during storage time (Izumi, 2012). Fish species differ in their optimal pH range. It is a suitable index for assessment of the freshness. pH values play an important role in the

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microbiological growth that affects the shelf life of the products (Hathout-Amal and Ali –Soher, 2010). The fish protein degraded during fish perishing. This produces an increase in typical substances, such as ammonia and volatile nitrogen compounds, whose concentrations are an index of the deterioration status. The TVN (Total Volatile Nitrogen) is a method of analysis that quantifies the presence of nitrogenous compounds (ammonia, dimethyl, and trimethyl amine) in fish, revealing the degree of freshness. Consumption of deteriorated fish can seriously affect consumer health. The rapid detection of freshness is a necessary task to evaluate and monitor the quality and safety of this valuable source of protein (Khoshnoudi-Nia and Moosavi-Nasab, 2019).

The current study was carried out to inspect the chemical profile of salted and smoked fish at retail markets.

2. MATERIAL AND METHODS

The Institutional Animals Care and Use Committee of the Faculty of Veterinary Medicine, Benha University approved the present study by Ethical Approval No. BUFVTM (13-02-23).

2.1. Collection of samples:

Ninety random samples of smoked herring, salted sardine, and fesiekh (30 each) have been collected from several markets at the Egyptian Gharbia governorate–at different times(2021-2022) . The collected samples have been submitted immediately to the laboratory for an expedited evaluation after being kept apart in an insulated ice box for the keeping quality tests (pH, TVB-N, and TMA and TBA), salt content, and heavy metal residues (mercuryand lead).

2.2. Keeping quality tests:

2.2.1. Determination of pH (Pearson, 2006):

In a blender, 10 g of the sample and 10 ml of neutralized distilled water were mixed. At room temperature, the homogenate was shaking constantly for ten minutes. The pH value was determined using an electrical pH meter (Bye model 6020, USA).

2,2.2. Determination of total volatile basic nitrogen "TVN" (ES: 63-9/ 2006):

Ten gm of sample and 300 ml of distilled water were added to a sterile distillation flask, and they were stirred with a polytron probe. Then antifoaming agent and 2 g of magnesium oxide were added. A 500-milliliter receiving flask was filled with 25 milliliters of 2 boric acid as indicator. After 10 minutes, the distillation flask reached boiling temperature and contin ued boiling for another 25 minutes. Then, titration of TVN in boric acid using H_2So_4 N 0.1. Consequently, this formula was used to calculate. TVN/l00g= (mls H_2So_4 N 0.1 for sample – ml H_2So_4 N 0.1 for Blank) x 14

2.2.3. Determination of trimethylamine (TMA):

Two milliliters of 0.05 M H_2So_4 were precisely added to the Conway dish's interior compartment, while two milliliters of the sample extract and one milliliter of saturated potassium carbonate (KCO_3) were added to the dish's outer ring. The dish was also covered and incubated at 36°C for two hours. Thus, H_2So_4 in the inner ring was titrated against 0.01M NaOH using methyl red indicator (T2 ml). TMA/l00g= 26.88 x (2-T_2) Where, T_2 = volume of NaOH consumed in the titration.

2.2.4. Determination of thiobarbituric acid number "TBA" (ES: 63-10/2006):

A distillation flask containing approximately 10 g of the examined fish sample, 50 ml of distilled water, 2.5 ml of diluted HCl, and 47.5 ml of water. Then tiny antifoaming agent particles were been added. The distillation flask was heated for a 50 ml distillation in less than 10 minutes after the boiling process began.r Consequently, a tube with a cover was filled with five ml of distilled solution and 5 ml of prepared thiobarbituric acid (which was generated by dissolving 0.2883 ml unthiobarbituric acid in trichloroacetic acid 90% and was finished to 100 ml). After being covered and submerged in a water bath, the tube was heated to boiling and left to cool in the water for ten minutes. then, the sample was assessed.

TBA value= absorbance of sample x 7.8 (malonaldehyde (mg/kg)

2.3. Determination of sodium chloride (AOAC, 2006):

Ten grams of the mixed material was combined with 40 ml of a 0.1N silver nitrate solution to correctly precipitate the chloride as silver chloride. There were also 20 ml of pure nitric acid supplied. The materials were then gradually heated at 60 °c for 15 minutes to dissolve them, except for silver chloride. Following cooling, 50 milliliters of distilled water and five milliliters of a saturated solution of ferric ammonium sulfate were added. The surplus silver nitrate was titrated against 0.1N ammonium thiocyanate using iron alum as an indicator. The amount of standard ammonium thiocyanate used in the titration is indicated by the number (R). Sodium chloride % = (ml of 0.1N silver nitrate -10 × 0.00585 × 10)

2.4. Determination of heavy metals (mercury and lead):

The amount of lead and mercury in the tested samples (mg/Kg) was calculated based on wet weight.

2.4.1. Washing procedures (AOAC, 2006):

Glassware and vessels were thoroughly cleaned with deionized water, immersed in hot, diluted HNO_3 (10%) for 24 hours, and then repeatedly rinsed with deionized water and dried to guarantee that no metal was present in any of the equipment.

2.4.2. Digestion technique (Tsoumboris and Papodoulou, 1994):

Accurately, 1 g of each sample was macerated by a sharp scalpel and digested by 10 ml of digestion mixture (60 ml of 65% Nitric acid and 40 ml of 70% perchloric acid) in screw-capped tube after maceration for determination of lead residues. Regarding mercury, 0.5 g of macerated sample was digested in 10 ml of concentrated H_2SO_4/ HNO_3 solution.

2.4.3. Preparation of blank and standard solutions (Shibamoto and Bjeldanes, 2000):

Blank and standard solutions were prepared in the same manner as applied for wet digestion and by using the same chemicals.

2.4.4. Analysis of mercury and lead concentrations:

The digest, blanks, and standard solutions were aspirated by Flame Atomic Absorption Spectrophotometer (VARIAN, Australia, model AA240 FS) and analyzed for mercury and lead concentrations.

2.5. Statistical Analysis:

All data were statistically analyzed according to Feldman et al. (2003), using SPSS 20 (SPSS, USA). The significance of the differences was determined by two-way ANOVA and the differences were considered significant at the $P < 0.05$ level.

3. RESULTS

The pH levels in table (1) of the tested smoked herring, salted sardine, and fesiekh were 6.18±0.03, 6.40±0.05, and 6.49±0.05, respectively. The acquired results showed that the approved samples were 29 (96.7%), 25 (83.3%), and 22 (73.3%) for smocked herring. Additionally, the acceptability of the investigated samples was recorded in accordance with the stated limit of 6.5 to EOS (2005). The TVB-N values in table (2) were 19.56 ±1.17, 24.08 ± 1.32 and 28.73 ± 1.49 (Mg/100g) in examined fesiekh, salted sardine, and smocked herring, respectively, The TVB-N values in table (2) were 19.56 ±1.17, 24.08 ± 1.32 and 28.73 ± 1.49 (Mg/100g) in examined fesiekh, salted sardine, and smocked herring, respectively.

Table 1 Acceptability of pH values for the examined samples of salted and smoked fishand their analytical results (n=30).

Fish products	pH	Mean ± S.E*	Accepted samples
Smoked herring		6.18± 0.03^{C}	29 (96.7%)
Salted sardine	6.0-6.5	6.40± 0.05 ^{AB}	25 (83.3%)
Fesiekh		6.49± 0.05^{A}	22 (73.3%)

* Means with different superscript letters were significantly different (P<0.05). *Egyptian Organization for Standardization (2005) No. 288/2005 for smoked fish (2005), No.1725/2005 for salted sardine (2005), No. 288/2005 for Fesiekh (2005)

Table (2) illustrated that, the TVB-N values in were 19.56 ±1.17, 24.08 ± 1.32 and 28.73 ± 1.49(Mg/100g) in examined fesiekh, salted sardine, and smocked herring, respectively.

Table 2 Analytical results and acceptability of TVB-N (mg/100g) for examined samples of salted and smoked fishand their analytical results (n=30).

Fish products	MRL (mg %)*	mean ± S.E*	Accepted samples
Smoked herring		19.56± 1.17 ^{C}	28 (93.3%)
Salted sardine	30	24.08± 1.32 ^{B}	23 (76.7%)
Fesiekh		28.73± 1.49 ^{A}	21 (70%)

* Means with different superscript letters are significantly different (P<0.05). *Egyptian Organization for Standardization (2005)

Furthermore, Table 3. Analytical results and acceptability of TMA (mg/100g) and TBA (mg/kg) in the examined salted and smoked fish were showed in table 3 and table 4 respectively.

Table 3 Analytical results and acceptability of TMA (mg/100g) for examined samples of salted smoked fish (n=30).

Fish products	MRL (mg %)*	mean ± S.E*	Accepted samples
Smoked herring		4.71± 0.39^{C}	27 (90%)
Salted sardine	10	6.98± 0.55^{B}	22 (73.3%)
Fesiekh		9.04± 0.67 ^{A}	20 (66.7%)

* In the same column, means with different superscript letters are significantly different (P<0.05). MRL refers to Maximum Residue levels

Table 4 The results and acceptability of TBA (mg/kg) in the examined samples of salted and smoked fish (n=30).

Fish products	MRL (mg/ Kg)*	mean ± S.E*	Accepted samples
Smoked herring		2.62± 0.14 ^{B}	29 (96.7%)
Salted sardine	4.5	3.36± 0.18^{AB}	24 (80%)
Fesiekh		3.54± 0.25 ^{A}	21 (70%)

* In the same column, means with different superscript letters are significantly different (P<0.05). *Egyptian Organization for Standardization (2005)

Table 5 revealed that, the acceptance rates were 27 (90%), 22 (73.3%), and 20 (66.0%) for the same analyzed samples, respectively Acceptability of Salt content (%) in the examined samples of salted and smoked fish.

Table 5 Acceptability of Salt content (%) in the examined samples of salted and smoked fish (n=30).

Fish products	MRL*	Mean ± S.E*	Accepted samples
Smoked herring		6.33± 0.02 ^{B}	23 (76.7%)
Salted sardine	Not less than 5%	6.87± 0.04 ^{AB}	26 (86.7%)
Fesiekh		7.15± 0.05 ^{A}	26 (86.7%)

* In the same column, means with different superscript letters are significantly different (P<0.05). *Egyptian Organization for Standardization (2010)

As shown in table 6 mercury concentrations in the smocked herring, salted sardine, and fesiekh samples were 0.46 ± 0.01, 0.72 ± 0.01, and 0.98 ± 0.02 mg/kg, respectively. Moreover, the lead level (mg/kg) were 0.25 ± 0.01, 0.41 ± 0.01and 0.46 ± 0.02 in smocked herring, salted sardine and fesiekh samples, respectively (table 7).

Table 6 Analytical results and acceptability of mercury residues (mg/Kg) in the examined samples of salted and smoked fish (n=30).

Fish products	MRL (mg/Kg)*	Mean ± S.E*	Accepted samples
Smoked herring		0.46 ± 0.01 ^{C}	27 (90%)
Salted sardine	0.5	0.72 ± 0.01 ^{B}	22 (73.3%)
Fesiekh		0.98 ± 0.02 ^{A}	21 (70%)

* In the same column, means with different superscript letters are significantly different (P<0.05). *Egyptian Organization for Standardization (2010)

Table 7 The results and acceptability of lead residues (mg/Kg) in the examined samples of smoked and salted fish (n=30).

Fish products	MRL (mg/Kg)*	Mean ± S.E*	Accepted samples
Smoked herring		0.25 ± 0.01 ^{B}	26 (86.7%)
Salted sardine	0.3	0.41 ± 0.01 ^{AB}	24 (80%)
Fesiekh		0.46 ± 0.02 ^{A}	23 (76.7%)

B* In the same column, means with different superscript letters are significantly different (P<0.05). *Egyptian Organization for Standardization (2005)

4. DISCUSSION

Fresh fish has a pH that is almost neutral and gradually rises with storage. Decomposition is indicated by pH levels above 7.1, pH used as a guide(*Özyurt, et al., 2009*). The pH is a key indicator of fish quality and can be utilized as a general rule. The pH levels in table (1) of the tested samplesof smoked herring, salted sardine, and fesiekh were 6.18± 0.03, 6.40 ±0.05, and 6.49± 0.05, respectively. Edriset al. (2014) recorded 6.39± 0.01 and 6.24 ±0.02 in examined fesiekh and salted sardine. Higher pH values were attained for the salted sardine and fesikh samples, 6.70± 0.01 and 6.90± 0.05, respectively El-Sheshnagui (2006). The acquired results showed that the approved samples were 29 (96.7%), 25 (83.3%) and 22 (73.3%) for smocked herring. Additionally, the acceptability of the investigated samples was recorded in accordance with the stated limit 6.5 of (EOS, 2005).

Total Volatile Basic Nitrogen (TVB-N) is one of the fish quality index's key parameters. Spoiled fish smell bad because of ammonia, monomethylamine, dimethylamine, and trimethylamine, which are produced by spoilage bacteria and endogenous enzymes. These compounds are linked to the growth of this organism, 35 to 40 mg of TVB-N per 100 g of fish muscle is generally considered to be an indication that the product is rotting according to Lakshmanan (2000).

The TVB-N values in table (2) were 19.56 ±1.17, 24.08 ± 1.32 and 28.73 ± 1.49(Mg/100g) in examined fesiekh, salted sardine, and smocked herring, respectively. The acceptability of the examined samples was recorded according to the established limit (30 mg %) of EOS (2005). The obtained results showed that the accepted samples were 28 (93.3%), 23 (76.7%), and 21(70%), and for smocked herring, salted sardine, and fesiekh, respectively.

Trimethylamine (TMA-N) is a crucial indicator of fish deterioration, particularly for marine fish. TMA-N comes from trimethyl amine oxide (TMAO), which is needed by marine fish for proper osmoregulation. According to Kilnice et al. (2008), during degradation, enzymes change TMAO to TMA. TMA-N accumulates quickly in muscle when kept refrigerated, making it a valuable tool for evaluating fish quality (Gökodlu et al., 1998).

The TMA values were 4.71±0.39, 6.98±0.55, and 9.04±0.67(mg/100g) for the examined salted sardine, salted herring, and fesiekh, respectively (Table 3). Additionally, the acceptance rates were 27 (90%), 22 (73.3%), and 20 (66.0%) for the same analyzed samples, respectively. TMA

concentrations were below the essential threshold (10 mg per 100 g) designated as a sign of fish product deterioration (European Commission, 1995).

Fish's muscle lipid composition, which typically has a high percentage of polyunsaturated fatty acids and is thus vulnerable to oxidative attack, is the main cause of its lower shelf life. The TBA index is used to quantify malonaldehyde (MDA), one of the breakdown products of lipid hydroperoxides generated during the oxidation process of polyunsaturated fatty acids (Gomes et al., 2003). Lipid oxidation produces MDA, which is recognized as a trustworthy indicator of fish meat freshness. According to Benside et al. (2014), TBA is considered a spoiling signal when fish are analyzed microbiologically and organoleptically during the storage period. Table (4) showed that the TBA values were 2.62±0.14, 3.36±0.18, and 3.54±0.2(mg/kg) for investigated salted sardine, salted herring, and fesiekh, respectively. The findings indicated that the following samples were accepted for the 29 (96.7%), 24 (80%), and 21 (70%). The investigated samples were found to be acceptable when measured against the prescribed limit of 4.5 mg/kg of EOS (2005).

According to the findings in table (5), the investigated smocking herring, salted sardine, and fesiekh, respectively, had salt content values of 6.87, 6.13, and 7.15 mg/kg, respectively. The obtained results indicated that the acceptable samples for smocked herring, salted sardine, and fesiekh, respectively, were 23 (76.7%) and 26(86.7%) based on the stipulated limit of 5% (EOS, 2005) for the salt content.

Due to their long-term persistence, toxicity, bioaccumulation, and biomagnification along the water, sediments, and aquatic food chain, which results in a decline in fish populations, heavy metals are regarded to be major pollutants to aquatic ecosystems. Compared to fresh and analytically salted fish, industrially salted fish had higher amounts of most of the metals (FAO 2003).

Regarding table (6) Mercury concentrations in the smocked herring, salted sardine, and fesiekh samples were found to be 0.46 ± 0.01, 0.72 ± 0.01, and 0.98 ± 0.02 mg/kg, respectively. According to the obtained data, salted sardines was 1.26±0.13, lower than that recorded by Latif (2018) and higher than that recorded by Sallam and El-Gazzar (1997), who reported 0.253±0.037. Regarding the analyzed samples' acceptability about the set limit of 0.5 mg/kg of (EOS,2005), the results obtained indicated that the accepted samples for the same examined samples were 21, (70 %), 22(73.3%), and 27 (90%), respectively.

The obtained results showed the concentrations of mercury were 0.46 ± 0.01, (0.72 ± 0.01 and0.98 ± 0.02 and mg/kg in Smocked herring, salted sardine and Fesiekh samples, respectively. The result of salted sardine was higher than that recorded by Sallam and El-Gazzar (1997) who recorded 0.253 ± 0.037 and lower than that recorded by Latif (2018) who was 1.26±0.13. Regarding the acceptability of the examined samples according to the established limit of 0.5 mg/ kg of EOS, (2005), the obtained results showed that the accepted samples were 27 (90%), 22 (73.7%) and 21(70%) for the same examined samples, respectively.

Lead is among the metals that most familiar for people. It gets into the aquatic system as a result of shallow soil erosion and air deposition. The results presented in **table (7)** showed that lead level (mg/kg) were 0.25 ± 0.01, 0.41 ± 0.01and 0.46 ± 0.02 in smocked herring, salted sardine and fesiekh samples, respectively. For sardine samples, these results were similar to those recorded by Yosef and Gomaa (2011)(0.448 to 1.226 mg/kg), while higher than that recorded by SalahEl-Dien et al. (2005) (5.951 mg/kg). Furthermore, the obtained results of the examined samples of smocked herring were nearly similar to those recorded by Morshady et al. (2013) (0.127 ± 0.02 mg/kg), while higher results were recorded by Celik and Oehlenschlager (2007) which were 0.076 to 0.314 mg/kg and the lower result obtained by Khansari et al. (2005) that recorded 0.0366 mg/kg. Accordingly, the acceptability of the examined samples was recorded according to the established limit of 0.3 mg/kg of EOS (2005), the obtained results showed that the accepted samples were 26 (86.7%), 24 (80 %) and 23 (76.7 %) for smocked herring, salted sardine and fesiekh samples, respectively.

5. CONCLUSIONS

Fish products that have been smoked and slated are customarily consumed in Egypt on a variety of occasions. There are safety concerns with these products when they come to salting or smoking. One of the main problems is limiting the quality and acceptability of certain fish products. There are definite changes that cause fish to lose some of their sensory qualities, develop an odd flavor, rancid taste, and lose muscle color, all of which detract from the fish's aesthetic appeal. They also cause additional compounds such as heavy metals that might be harmful to human health. Accordingly, using of good quality fresh fish for the manufacture of smoked and salted fish products besides the application of good hygienic measures during their processing is of great significance for human safety.

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