

5. SUMMARY

The current study was conducted with the aim of evaluating the quality and safety of Mullet fish obtained from two farms in Fayoum Governorate and its smoked products; with a focus on microbial and chemical contaminants. The two farms (A) and (B) are surrounding by Qarun Lake. One farm is irrigated from Al-Batts drains and the other from El-Wadi drains.

Accordingly, the objectives of the present investigation can be summarized in the following points:

1. Determination the chemical composition and quality properties of fresh Mullet fish obtained from the two farms (A) an (B).
2. Assessment the safety of Mullet fish obtained from the above mentioned farms by the determination of chemical contaminants of biogenic amines and heavy metals as well as microbiological loads of total bacterial count and coliform bacterial counts.
3. Evaluating the quality attributes of cold and hot smoked Mullet fish obtained from the two farms through determination of the chemical composition and physicochemical properties and evaluating the sensory quality characteristics.
4. Assessment the safety of the smoked Mullet fish by determination of the chemical contaminants of biogenic amines, polycyclic aromatic hydrocarbons and heavy metals. The microbial contamination of the smoked samples were also assayed.
5. Studying the storage stability of the smoked Mullet fish during refrigerated storage at $3\pm1^{\circ}\text{C}$ by following up changes in chemical composition, physicochemical quality parameters, chemical contaminants of biogenic amines, polycyclic aromatic hydrocarbons and heavy metals, microbiological loads. Sensory evaluation of the stored smoked samples was also conducted.

The obtained results could be summarized in the following points:

5.1. Raw Mullet Fish

5.1.1. Chemical Composition:

The chemical compositions for the fish sample obtained from farm (A) showed that moisture, protein, lipids, ash, and carbohydrates were 74.82, 19.59, 4.28, 1.23 and 0.08 %; respectively; while the corresponding values for sample from farm (B) were 72.10%, 19.34 %, 7.38 %, 1.11 % and 0.07%, respectively (on wet weight basis).

5.1.2. Physiochemical Quality Parameters:

pH value, total volatile basic nitrogen (TVBN), trimethylamine nitrogen (TMA-N) and thiobarbituric acid (TBA) are good indexes to determine the freshness and quality of fish and fish products.

5.1.2.1. pH value

The results indicated that pH value of fresh Mullet fish obtained from farms (A) and (B) samples were 6.27 and 6.10, respectively.

5.1.2.2. Total volatile basic nitrogen (TVB-N)

The results showed that TVB-N values of fresh Mullet fish obtained from farms (A) and (B) were 15.12 and 12.65 mg/100g sample, respectively.

5.1.2.3. Trimethylamine nitrogen (TMA-N)

Data showed that the values of TMA for fresh Mullet fish obtained from farms (A) and (B) were 0.66 and 0.48 mg/100g, respectively.

5.1.2.4. Thiobarbituric acid (TBA)

Data indicated that TBA values of fresh Mullet fish obtained from farms (A) and (B) were 0.52 and 0.35 mg MA/kg, respectively.

5.1.3. Biogenic Amines (BAs)

The results showed that Mullet fish from farm (A) contains a relatively higher concentrations of histamine (16.00 mg/kg), followed by tyramine (10.90 mg/kg), cadaverine (9.08 mg/kg), putrescine (5.22 mg/kg)

and spermine (2.90 mg/kg). While these biogenic amines; cadaverine, histamine, tyramine, putrescine and spermine were determined by 8.80, 8.00, 5.60, 1.01 and 0.74 mg/kg, respectively in in fresh Mullet fish from farm (B).

5.1.4. Heavy metals

The chemical analysis indicated that the heavy metals; lead (Pb), cadmium (Cd) and zinc (Zn) concentrations in raw Mullet fish from farm (A) were 0.423, 0.215 and 1.120 (ppm), respectively. While the values of these metals in Mullet fish from farm (B) were 0.310, 0.120 and 0.880 (ppm); respectively.

5.1.5. Microbial Aspects

The results indicated that (TBC), (TCBC) and (Y and M) counts were 4.26, 3.04 and 1.88 log₁₀cfu/g in fresh Mullet fish flesh obtained from farm (A), and 3.36, 2.48 and 1.54 log₁₀cfu/g in fresh Mullet fish flesh obtained from farm (B).

5.2. Smoked Mullet Fish:

5.2.1. Effect of smoking methods on chemical composition of Mullet fish

Data showed that moisture content of fresh Mullet fish from farm (A) considerably decreased from 74.82% to 63.22 and 59.32% in cold and hot smoked Mullet fish from farm (A), respectively. Similarly, moisture content of fresh Mullet fish from farm (B) decreased from 72.10% to 61.25 and 58.06% in cold and hot smoked samples, respectively.

The chemical analysis showed that protein, fat, ash, carbohydrates and salt (NaCl) contents of fresh Mullet fish from farm (A) were 19.59, 4.28, 1.23 and 0.08% (on wet basis), respectively. These values increased to 24.75, 6.62, 5.14, 0.27 and 3.17%, respectively in cold smoked samples and to 26.84, 7.91, 5.61, 0.32 and 3.70%, respectively in hot smoked

samples. Similarly, protein, fat, ash, carbohydrates and salt (NaCl) contents of fresh Mullet fish from farm (B) increased from 19.34, 7.38, 0.07 and 1.11%, respectively to 25.27, 7.82, 5.61, 0.44 and 3.35%, respectively in cold smoked samples and increased to 27.42, 7.50, 6.52, 0.50 and 4.05%, respectively in hot smoked samples.

5.2.2. Effect of smoking methods on physiochemical quality parameters of smoked Mullet fish

Total volatile basic nitrogen (TVB-N), Trimethylamine nitrogen (TMA-N) and Thiobarbituric acid (TBA) were determined and pH was also measured in smoked Mullet fish.

5.2.2.1. pH value

The results given showed that pH value of raw Mullet fish from farm (A) was 6.27 and decreased to 6.08 and 6.13 for cold and hot smoked samples, respectively. Also, pH value of raw Mullet fish obtained from farm (B) decreased from 6.10 to 5.92 and 6.02 for cold and hot smoked fish samples, respectively.

5.2.2.2. Total volatile basic nitrogen (TVB-N)

The results showed that TVB-N of fresh fish from farm (A) significantly increased from 15.12 mg/100g to 21.88 and 18.16 mg/100g in cold and hot smoked samples, respectively. Similarly, TVB-N value of raw Mullet fish obtained from farm (B) increased from 12.65 mg/100g to 16.30 and 15.16 mg/100g of cold and hot smoked fish samples, respectively.

5.2.2.3. Trimethylamine nitrogen (TMA-N)

The results showed that TMA-N contents of raw Mullet fish obtained from farm (A) as well as the cold smoked and hot smoked products were determined by 0.66, 0.88 and 0.72 mg/100g, respectively. Also, TMA-N of raw Mullet fish from farm (B) increased from 0.48 mg/100g to 0.68 and 0.60 mg/100g of cold and hot smoked fish samples, respectively.

5.2.2.4. Thiobarbituric acid (TBA)

TBA value of raw Mullet fish from farm (A) was 0.52 mg malonaldehyde (MAD)/kg and increased to 0.68 and 0.88 mg MAD/kg of cold and hot smoked fish samples, respectively. While, TBA value of raw Mullet fish from farm (B) was 0.35 mg MAD/kg and increased to 0.51 and 0.63 mg MAD/kg of cold and hot smoked fish samples, respectively.

5.2.3. Effect of smoking methods on biogenic amines of smoked Mullet fish

The results showed fresh fish from farm (B) was characterized by lower contents of the biogenic amines than in fish from farm (A) with total concentrations of 24.15 and 44.10 mg/100g, respectively.

It was found that histamine, tyramine and cadaverine, are the predominant amines in the fresh fish samples obtained from farm (A) with the concentrations of 16.00, 10.90 and 9.08mg/100g followed by putrescine and spermine with concentrations of 5.22 and 2.90 mg/100g, respectively. After smoking process, cadaverine, histamine, putrescine, tyramine, and spermine were determined by (17.45 and 12.00 mg/100g), (12.07 and 6.08 mg/100g), (8.90 and 6.52 mg/100g), (6.72 and 4.41 mg/100g) and (5.45 and 3.00 mg/100g) in cold and hot smoked samples, respectively.

Also, the results showed that the concentrations of cadaverine, histamine and tyramine, are the predominant amines in the fresh fish samples obtained from farm (B) were 8.80, 8.00 and 5.60 mg/100g, respectively followed by putrescine and spermine with concentrations of 1.01 and 0.74 mg/100g, respectively. After smoking process, cadaverine, tyramine, putrescine, histamine and spermine were determined by (11.40 and 9.12 mg/100g), (4.80 and 3.45 mg/100g), (3.33 and 1.84 mg/100g),

(3.00 and 1.23 mg/100g) and (2.71 and 1.50 mg/100g) in cold and hot smoked samples, respectively.

5.2.4. Effect of smoking methods on polycyclic Aromatic Hydrocarbons (PAHs) in smoked Mullet fish

It was found that 5 compounds of PAHs; acenaphthylene, acenaphthene, fluorene, fluoranthene and pyrene were detected in both cold and hot smoked fish samples from the two farms (A) and (B), while phenanthrene and anthracene were detected only in cold smoked samples from farm (A) and (B). Benzo (a) pyrene (BaP) compound that considered as indicator for carcinogenic PAHs was not detected in both cold and hot smoked Mullet fish. Also, PAH4; benzo (a) pyrene, benzo (a) anthracene, benzo (b)fluoranthene and chrysene did not detected in both the cold and hot smoked Mullet fish. It was noticed the higher levels of PAHs compounds were found in the cold smoked samples from farms (A) and (B) than in the hot smoked samples and also in smoked samples obtained from farm (A) than that in smoked samples obtained from farm (B).

The total PAHs in the cold and hot smoked Mullet fish samples obtained from farm (A) were 42.9 and 12.1 $\mu\text{g/kg}$, respectively, while in cold and hot smoked samples obtained from farm (B) we 32.1 and 11.2 $\mu\text{g/kg}$, respectively.

5.2.4.1. Toxic Equivalent Factors (TEFs) and B [a] P Equivalent of PAHs found in smoked mullet fish samples

The results showed The toxic equivalent factors (TEFs) and B [a] P Equivalent of PAHs in cold and hot smoked mullet fish obtained from the two fish farms (A and B). After cold and hot smoking of farm (A) the B [a] P Equivalent of Acenaphthylene, Acenaphthene, Fluorene, Anthracene, Phenanthrene, fluoranthene and Pyrene were 0.0043, 0.0038, 0.0067, 0.031, 0.0092, 0.0025 and 0.0124 and total B [a] P Equivalent was 0.069 in cold smoked samples respectively, In the case of hot smoked samples obtained from farm (A), the values of Acenaphthylene, Acenaphthene,

Fluorene, fluoranthene and Pyrene were 0.0022, 0.0015, 0.0027, 0.0019, 0.0038 and total B [a] P Equivalent was 0.0121, respectively.

On the other side, B [a] P Equivalent of Acenaphthylene, Acenaphthene, Fluorene, Anthracene, Phenanthrene, fluoranthene and Pyrene for smoked products of farm (B) were 0.0025, 0.0035, 0.0042, 0.019, 0.007, 0.0035 and 0.0095 and the total B [a] P Equivalent was 0.0492 in cold smoked samples, respectively. Also, the values of Acenaphthylene, Acenaphthene, Fluorene, fluoranthene and Pyrene were 0.0017, 0.0014, 0.002, 0.0014 and 0.0047 and the total B [a] P Equivalent was 0.0112 for hot smoked samples obtained from farm (B), respectively.

5.2.4.2. Molecular weight of PAHs in smoked Mullet fish

The total concentration of the low molecular weights (LWM) of PAHs was higher than the medium molecular weights (MMW) in both smoked fish farms samples. LMW levels in cold samples obtained from farm (A) were the highest value in all samples, recorded by 28 $\mu\text{g} / \text{kg}$ followed by cold smoked samples from farm (B) recorded by 19.1 $\mu\text{g} / \text{kg}$, while hot smoked samples recorded 12.8 and 5.1 $\mu\text{g} / \text{kg}$ for farm (A) and (B) respectively. Generally cold smoked samples in both farms contained higher levels of LMW and MMW compounds.

5.2.4.3. Category of PAH concentration in cold and hot smoked Mullet fish

Concentrations of PAHs were 42.9 and 12.1 $\mu\text{g}/\text{kg}$ in cold and hot smoked fish from farm (A), respectively after smoking and were 32.1 and 11.2 $\mu\text{g}/\text{kg}$ in cold and hot samples from farm (B), respectively.

5.2.5. Heavy metals in fresh and smoked Mullet fish

The concentration of Pb in raw Mullet fish from farms (A) and (B) were 0.103 and 0.052 ppm; respectively. After smoking process the concentrations of Pb increased to 0.210 and 0.252 ppm in cold and hot smoked fish samples from farm (A), respectively, and increased to 0.190

and 0.210 ppm, respectively in cold and hot smoked samples from farm (B).

The level of Zn in Mullet fish from farm (A) increased from 1.120 ppm to 1.760 and 1.982 ppm in cold and hot smoked samples, respectively. Also, concentration of Zn increased from 0.880 ppm in fresh raw Mullet fish to 1.055 and 1.750 ppm in the cold and hot smoked samples from farm (B); respectively.

The initial concentrations of Cd in fresh raw fish from the farms (A) and (B) were determined by 0.215 and 0.120 ppm; respectively. After smoking, the levels of Cd were 0.215, 0.109 and 0.170 ppm in raw, cold and hot smoked samples from farm (A), respectively and were 0.120, 0.0750 and 0.090 ppm in fresh, cold smoked and hot smoked samples from farm (B); respectively.

5.2.6. Microbial aspects of fresh and smoked Mullet fish

5.2.6.1. Total bacterial count (TBC)

The results given showed that the smoking procedures caused a considerable reduction of total bacterial count (TBC) in the smoked fish samples. TBC of raw Mullet fish sample obtained from farm (A) decreased from 4.26 Log₁₀cfu/g to 3.35 and 3.05 log₁₀cfu/g of cold and hot smoked fish samples, respectively. Also, TBC of raw Mullet fish sample from farm (B) decreased from 3.36 log₁₀cfu/g to 3.12 and 2.85 Log₁₀cfu/g of cold and hot smoked fish samples, respectively.

5.2.6.2. Coliform bacterial count (TCBC)

Data showed that coliform count was 2.84 log₁₀cfu/ g for fresh Mullet fish from farm (A) and decreased to 1.64 and 1.22 log₁₀cfu/g of cold and hot smoked fish samples, respectively. Also, (TCBC) for fresh Mullet fish from farm (B) decreased from 2.00 log₁₀cfu/g to 1.2 and 1.00 log₁₀cfu/g of cold and hot smoked samples, respectively.

5.2.6.3. Yeast and mold counts

The results showed that the counts of yeasts and molds in fresh Mullet fish from farm (A) decreased from 1.88 to 1.20 and 0.82 log₁₀cfu/g of cold and hot smoked fish samples, respectively and from 1.54 log₁₀cfu /g in fresh samples from farm (B) to 1.00 and 0.50 log₁₀cfu/g of cold and hot smoked fish samples, respectively.

5.2.7. Effect of smoking process on sensory quality attributes of smoked Mullet fish

The values for color, taste, odor, texture and overall acceptability of cold smoked Mullet fish obtained from farm (A) were 8.0, 7.5, 8.0, 7.5 and 7.5, respectively. The score values for the same parameters for the hot smoked Mullet fish obtained from the same farm (A) were 9.0, 8.5, 9.0, 8.6 and 8.8, respectively. The results also showed that score values of color, taste, odor, texture and overall acceptability of cold smoked Mullet fish obtained from farm (B) were 8.5, 8.0, 8.0, 8.5 and 8.3, respectively and 9.2, 9.5 9.2, 9.0 and 9.2, respectively for the hot smoked Mullet fish obtained from farm (B).

5.3. Refrigerated storage of smoked Mullet fish at 3±1°C

The storage stability of cold and hot smoked Mullet fish was investigated by refrigerated storage at 3±1°C for 20-35 days and the samples were withdrawn at intervals of five days to determine the effects of coldstorage on chemical compositions, physiochemical quality attributes, biogenic amine (BAs), Polycyclic aromatic hydrocarbons (PAHs) and heavy metals. Microbiological examination was assessed and quality sensory properties were evaluated.

5.3.1. Effect of refrigerated storage on chemical composition of smoked Mullet fish

5.3.1.1. Moisture content

The results showed that the moisture contents of cold and hot smoked Mullet fish samples obtained from farm (A) were determined by 63.22% and 59.32 % at zero time of storage, respectively. During storage, these values gradually (significantly $P > 0.05$) decreased down to 60.20 and 56.85% at the end of 20 and 35days storage, respectively. Also, moisture contents of the cold and hot smoked Mullet fish samples obtained from farm (B) showed similar changes. Moisture contents of cold and hot smoked Mullet fish obtained from farm (B) decreased from 61.25 and 58.06% at zero time of storage to 58.70 and 55.60% at the end of 25 and 35 days storage, respectively.

5.3.1.2. Protein content

Protein content of smoked Mullet fish samples obtained from farm (A) immediately after smoking were determined by 24.75 and 26.84% for cold and hot smoked Mullet fish samples, respectively. At the end of storage period of cold and hot smoked samples of 20 and 35 days, respectively, protein content significantly ($P > 0.05$) decreased to 21.50 and 23.60%, respectively. Similarly, protein contents of cold and hot smoked Mullet fish samples obtained from farm (B) significantly ($P > 0.05$) decreased from 25.27 and 27.42% at zero time to 22.13 and 24.21 %, respectively at the end of 25 and 35days storage at $3\pm 1^{\circ}\text{C}$.

5.3.1.3. Lipids content

Fat contents of cold and hot smoked Mullet fish samples obtained from farm (A) after smoking were 6.62 and 7.91% respectively. By ending the storage period of cold and hot smoked samples at 20 and 35 days, respectively, these values significantly ($P > 0.05$) decreased to 5.16 and 6.93%, respectively. Similarly, fat contents of cold and hot smoked Mullet

fish obtained from farm (B) significantly ($P > 0.05$) decreased from 7.82 and 7.50% at zero time of storage to 6.46 and 6.50%, respectively at the end of 25 and 35days storage.

5.3.1.4. Ash content

Ash contents of cold and hot smoked Mullet fish samples obtained from farm (A) significantly ($P < 0.05$) increased from 5.14 and 5.61% to 12.09 and 11.49%, respectively at the end of 20 and 35days of storage, respectively. The same findings were also found in ash contents of cold and hot smoked fish samples obtained from farm (B). Ash contents of the cold and hot smoked samples obtained from farm (B) significantly ($P < 0.05$) increased from 5.22 and 6.52% at zero time of storage to 11.59 and 12.47%, respectively at the end of 25 and 35days storage; respectively.

5.3.1.5. Total carbohydrates

The results showed that total carbohydrates of smoked Mullet fish samples obtained from farm (A) after smoking were 0.27 and 0.32% for cold and hot smoked Mullet fish, respectively. These values gradually increased to 1.05 and 1.13%, respectively at the end of 20 and 35days storage. Also, carbohydrate contents of smoked Mullet fish obtained from farm (B) showed similar changes during refrigerated storage. Carbohydrate contents of cold and hot smoked Mullet fish gradually increased from 0.44 and 0.50% at zero time of storage to 1.12 and 1.22 %, respectively at the end of 25 and 35days storage.

5.3.1.6. Sodium chloride (NaCl)

Sodium chloride contents of smoked Mullet fish samples obtained from farm (A) immediately after smoking were 3.54 and 4.21% for cold and hot smoked Mullet fish, respectively and significantly ($P < 0.05$) increased to 9.03 and 9.19%, respectively at the end of 20 and 35days storage. Also, sodium chloride contents of cold and hot smoked Mullet fish from farm (B) gradually increased from 3.32 and 5.02% at zero time of

storage to 9.10 and 9.97%, respectively at the end of 25 and 35 days of refrigerated storage.

5.3.2. Effect of refrigerated storage on physiochemical quality parameters of smoked Mullet fish

The physiochemical quality attributes are commonly used to assessing the quality as well as the shelf life of fish and fish products. pH value, total volatile basic nitrogen (TVB-N), Trimethyl amine nitrogen (TMA-N) and thiobarbituric acid (TBA) are the major factors that affect the overall acceptability of smoked fish products for human consumption.

5.3.2.1. pH value

The pH values of cold and hot smoked Mullet fish samples from farm (A) were measured by 6.08 and 6.13 at zero time of cold storage. These values significantly ($P < 0.05$) increased to 6.82 and 6.73 for cold and hot smoked samples, respectively at the end of 20 and 35days storage. Similarly, the pH values for cold and hot smoked samples from farm (B) increased from 5.97 and 6.06 at zero time of storage to 6.75 and 6.67, respectively at the end of 25 and 35 days storage, respectively.

5.3.2.2. Total volatile bases nitrogen (TVB-N)

TVB-N contents of cold and hot smoked Mullet fish from farm (A) were determined by 18.16 and 21.88 mg/100g, respectively at zero time of storage. During storage, these values gradually increased (significantly, $P < 0.05$) to 32.20 and 28.84 mg/100g, respectively at the end of 20 and 35days storage, respectively. The TVB-N values for cold and hot smoked samples from farm (B) showed similar changes, the values significantly ($P < 0.05$) increased from 15.16 and 16.30 mg/100g at zero time of storage to 30.84 and 26.50 mg/100g for cold and hot smoked samples, respectively at the end of 25 and 35days storage, respectively.

5.3.2.3. Trimethyl amine nitrogen (TMA-N)

TMA-N contents of cold and hot smoked Mullet fish samples from farm (A) were determined by 0.72 and 0.88 mg/100g (w.w.), respectively at zero time of storage and significantly ($P < 0.05$) increased to 4.22 and 3.98 mg/100g at the end of 20 and 35 days of storage. For fish samples obtained from farm (B); the TMA-N contents of the smoked samples also showed similar trends. TMA-N contents of cold and hot smoked samples increased from 0.60 and 0.68 mg/100g at zero time of storage to 4.05 and 3.50 mg/100g, respectively at the end of 25 and 35 of days of storage.

5.3.2.4. Thiobarbituric acid (TBA) value

TBA values of cold and hot smoked Mullet fish samples from farm (A) determined as malonaldehyde were 0.68 and 0.80 mg MA/kg (w.w.), respectively at zero time of storage and significantly ($P < 0.05$) increased up to 3.50 and 3.08 mg MA/kg at the end of 20 and 35 days storage. Also, TBA values for cold and hot smoked samples from farm (B) showed similar changes from 0.41 and 0.52 mg MA/kg at zero time of storage and increased up to 3.22 and 2.95 mg MA/kg for cold and hot smoked samples, respectively at the end of 25 and 35 days storage.

5.3.3. Effect of refrigerated storage on biogenic amine (BAs) contents of smoked Mullet fish

The results showed that biogenic amines; spermine, putrescine, cadaverine, tyramine and histamine, of cold smoked Mullet fish from farm (A) were 5.45, 8.90, 17.45, 6.72 and 12.07 mg/kg, respectively at zero time of refrigerated storage. By ending the storage time of cold smoked sample after 20 days these values became 7.80, 13.25, 10.20, 4.34 and 17.80 mg/kg, respectively. Also, the determined biogenic amines in hot smoked Mullet fish from farm (A) at zero time of storage were 3.0, 6.52, 12.0, 6.80 and 4.41 mg/kg. As the storage time of hot smoked sample was extended to 35 days, at this time of storage the biogenic amines;

spermine, putrescine, cadaverine, tyramine and histamine were determined by 4.50, 7.13, 7.60, 11.20 and 2.90 mg/kg, respectively. The results also showed that the total biogenic contents of cold and hot smoked Mullet fish from farm (A) were 50.59 and 32.73 mg/kg at zero time of storage increased to 53.39 and 33.33 mg/kg at the end of storage periods.

The determined biogenic amines; spermine, putrescine, cadaverine, histamine, tyramine in cold smoked Mullet fish from farm (B) were 2.71, 3.33, 11.40, 3.00 and 4.80 mg/kg at zero time of storage. At the end of storage period of 25 days spermine, putrescine and histamine increased to 5.30, 8.50 and 11.3 and 2.33 mg/kg a, respectively while cadaverine and tyramine decreased to 8.5 and 2.33 mg/kg, respectively.

Also, in the hot smoked Mullet fish samples from farm (B) at zero time of storage, the contents of spermine, putrescine and histamine were 1.50, 1.84, and 1.23 mg/kg, respectively and increased to 3.40, 5.07 and 7.85 mg/kg, respectively while cadaverine and tyramine decreased from 9.12 and 3.45 mg/kg to 6.3 and 1.63 mg/kg, respectively at the end of storage 35 days of cold storage. The total values of BAs for cold and hot smoked Mullet fish from farm (B) were 25.24 and 17.14 mg/kg at zero time of storage increased to 35.93 and 24.25 mg/kg at the end of storage.

5.3.4. Effect of refrigerated storage on polycyclic aromatic hydrocarbons (PAHs) contents of smoked Mullet fish

The results showed that the polycyclic aromatic hydrocarbon PAHs compounds; acenaphthylene, acenaphthene, fluorene, anthracene, phenanthrene, fluoranthene and pyrene were determined in the cold smoked sample at zero time at the levels of 4.3, 3.8, 7.6, 3.1, 9.2, 2.5 and 12.4ug/kg; respectively. At the end of 20 days storage these values were decreased to 3.5, 2.0, 4.6, 2.6, 5.4, 2.0 and 8.8 ug/kg; respectively. Also, at zero time of storage of hot smoked sample, acenaphthylene, acenaphthene, fluorene, fluoranthene and pyrene were detected at the levels of 2.2, 1.5,

2.7, 1.9 and 3.8 ug/kg; respectively, while, anthracene and phenanthrene were not detected. By ending storage time at 35 days, the levels of the above detected PAHs compounds decreased to 1.5, 2.0, 1.5 and 2.5 ug/kg; respectively, while acenaphthene as well as anthracene and phenanthrene were not detected. Moreover, the total contents of PAHs were decreased from 42.9 to 28.9 ug/kg in cold smoked and from 12.1 to 8.8 ug/kg in hot smoked sample.

Almost, the same trend was also observed in the PAHs compounds in the smoked samples obtained from farm (B). The cold and hot smoked samples from farm (B) contained lower contents of the PAHs compounds in comparison with their contents in the smoked samples from farm (A). The PAHs compounds: acenaphthylene, acenaphthene, fluorene, anthracene, phenanthrene, fluoranthene and pyrene in cold smoked sample were decreased from 2.5, 3.5, 4.2, 1.9, 7.0, 3.5 and 9.5 ug/kg; respectively at zero time to 2.2, 2.8, 4.0, 1.5, 3.8, 2.3 and 6.3 ug/kg; respectively at the end of 25 days of cold storage. The initial values of all the different compounds were very low in the hot smoked sample. At the end of 35 days of storage, the contents of acenaphthene, fluorene, fluoranthene and pyrene were decreased from 1.4, 2.0, 1.4 and 4.7 ug/kg; respectively at zero time to 1.0, 1.6, 1.2 and 2.9 ug/kg, respectively. Acenaphthylene and phenanthrene 1.7 and 1.9 ug/kg; respectively and did not detected at the end of storage time and also anthracene was not detected in the hot smoked sample.

5.3.4.1. Toxic Equivalent Factors (TEFs) and B [a] P Equivalent of PAHs in smoked mullet fish

The toxic equivalent factors (TEFs) and B [a] P Equivalent of PAHs in the smoked Mullet fish samples obtained from the two fish farms at the end of refrigerated storage were estimated. The (B(a)P) Equivalent of Acenaphthylene, Acenaphthene, Fluorene, Anthracene, Phenanthrene,

Fluoranthene and Pyrene were 0.0035, 0.002, 0.0046, 0.026, 0.0054, 0.002, and 0.0088, respectively and the sum of B (a) P Equivalents Σ (BaPeqi) was 0.0523 in cold smoked fish obtained from farm (A) after 20 days of cold storage. On other side these values were 0.0015, ND, 0.002, ND, ND, 0.0015 and 0.0025, respectively and the sum of B [a] P Equivalents Σ (BaPeqi) was 0.0075 in hot smoked product from the same farm after 35 days of cold storage.

Data also show the B [a] P Equivalent of PAHs for smoked Mullet fish samples obtained from farm (B). At the end of 25 days of cold storage, BaPeqi of cold smoked products were 0.0022, 0.0028, 0.004, 0.015, 0.0038, 0.0023 and 0.0063 for Acenaphthylene, Acenaphthene, Fluorene, Anthracene, Phenanthrene, Fluoranthene and Pyrene, respectively while the sum of B [a] P Equivalents Σ (BaPeqi) was 0.0364. The values of these were ND, 0.001, 0.0016, ND, 0.0019, 0.0012 and 0.0029, respectively and Σ (BaPeqi) was 0.0086 in hot smoked products after 35 days of cold storage.

5.3.4.2. Total mean concentration ($\mu\text{g/kg}$) of PAHs in cold and hot smoked Mullet fish during cold storage according to their molecular weights

The total concentration of the low molecular weights (LMW) polycyclic aromatic hydrocarbons were (18.1 and 14.3 $\mu\text{g/kg}$) in cold smoked fish samples from both (A) and (B) fish farms, respectively. While, the LMW concentrations of hot smoked samples were 3.5 and 4.2 $\mu\text{g/kg}$ for both (A) and (B) fish resources, respectively. On other hand, the medium molecular weight compounds concentrations were 10.8 and 8.6 $\mu\text{g/kg}$ for farm (A) and (B) cold smoked products. While these values were 4 and 4.1 $\mu\text{g/kg}$ in hot smoked products, respectively.

5.3.4.3. Category of PAHs concentration ($\mu\text{g/kg}$) in the studied cold and hot smoked Mullet fish

The results showed that the categories of PAHs concentrations of smoked Mullet fish samples obtained from the two farms. ΣPAHS of cold smoked products of farms (A) and (B) were 28.9 and 22.9 $\mu\text{g/kg}$, respectively and classified as minimally contaminated. While the PAHS concentrations in hot smoked products of both farms (A) and (B) were 8.8 and 8.6 $\mu\text{g/kg}$, respectively and classified as not contaminated ($<10 \mu\text{g/kg}$).

5.3.5. Changes in heavy metals contents of smoked Mullet fish during cold storage at $3 \pm 1^\circ\text{C}$

5.3.5.1. Lead (Pb)

Lead (Pb) contents of cold and hot smoked fish samples obtained from farm (A) were 0.210 and 0.252 ppm, respectively and gradually increased ($P<0.05\%$) to 0.315 and 0.385 ppm at the end of 20 and 35 days storage, respectively. Similar observations were found in Pb concentrations in the smoked Mullet fish obtained from farm (B). The concentrations of lead in cold and hot smoked samples increased from 0.190 and 0.210 ppm, respectively at zero time of storage to 0.305 and 0.400 ppm, respectively at the end of 25 and 35 days.

5.3.5.2. Cadmium (Cd)

The levels of (Cd) in cold and hot smoked Mullet fish samples obtained from farm (A) were 0.109 and 0.170 ppm, respectively. These values of were gradually increased ($P<0.05$) up to 0.180 and 0.290 ppm at the end of 20 and 35 days storage, respectively. Also, (Cd) contents of smoked Mullet fish obtained from farm (B) increased from 0.075 and 0.090 ppm at zero time to 1.98 and 0.268 ppm at the end of 25 and 35 days storage, respectively.

5.3.5.3. Zinc (Zn)

Zn concentrations in cold and hot smoked Mullet fish samples obtained from farm (A) were determined by 1.760 and 1.982 ppm, respectively at zero time of refrigerated storage.

During storage, these values were gradually ($P < 0.05\%$) increased to 2.950 and 4.150 ppm at the end of 20 and 35 days the end of storage, respectively. Similarly, the values of Zn in cold and hot smoked Mullet fish samples obtained from farm (B) increased from 1.055 and 1.750 ppm at zero time to 3.100 and 4.000 ppm at the end of 25 and 35 days storage, respectively.

5.3.6. Microbiological aspects of smoked Mullet fish during refrigerated storage at $3 \pm 1^\circ\text{C}$

Cold and hot smoked processed from Mullet fish obtained from the two farms (A) and (B) were examined microbiologically for total bacterial count (TBC), coliform bacteria count (TCBC) as well as yeast and mold count (Y&M) during refrigerated storage at $3 \pm 1^\circ\text{C}$.

5.3.6.1. Total bacterial count (TBC)

The obtained results indicated that total bacterial counts (TBC) of cold and hot smoked Mullet fish samples obtained from farm (A) were assessed by 3.35 and 3.05 $\log_{10}\text{cfu/g}$, respectively. These values were gradually (significantly at $P < 0.05\%$) increased to 5.05 and 5.15 $\log_{10}\text{cfu/g}$ at the end of 20 and 35 days the end of storage, respectively. Also, TBC contents of smoked Mullet fish obtained from farm (B) were increased (significantly at $P < 0.05\%$) from 3.12 and 2.85 $\log_{10}\text{cfu/g}$ at zero time of storage to 5.00 and 4.95 $\log_{10}\text{cfu/g}$ at the end of 25 and 35 days storage of cold and hot smoked fish samples, respectively.

5.3.6.2. Coliform bacterial count (TCBC)

The values of total coliform bacterial counts (TCBC) of cold and hot smoked Mullet fish samples obtained from farm (A) were 2.64 and 2.24

log₁₀cfu/g, respectively at zero time of storage and gradually increased (significantly at P<0.05%) to 3.10 and 3.15 log₁₀cfu/g at the end of 20 and 35 days storage, respectively. Similarly, TCBC of smoked Mullet fish obtained from farm (B) significantly (P<0.05%) increased from 1.20 and 1.00 log₁₀cfu/g at zero time of storage to 3.05 and 3.00 log₁₀cfu/g at the end of 25 and 35days storage, respectively.

5.3.6.3. Yeasts and molds count (Y&M)

Yeasts and molds counts (Y&M) of smoked Mullet fish samples obtained from farm (A) immediately after smoking were determined by 1.20 and 0.82 log₁₀cfu/g, respectively for cold and hot smoked Mullet at zero time of storage. During storage, these values of (Y&M) were decreased to 1.05 and 0.60 log₁₀cfu/g, respectively after 5 days of storage and then gradually increased up to 3.55 and 3.85 log₁₀cfu/g at the end of storage period (20 and 35days, respectively). Yeasts and molds count (Y&M) counts of smoked Mullet fish obtained from farm (B) showed similar trends. Yeasts and molds counts were decreased from 1.10 and 0.50 log₁₀cfu/g at zero time of storage to 0.85 and 0.40 log₁₀cfu/g, respectively after 5 days of storage and then gradually increased up to 3.40 and 3.35 log₁₀cfu/g at the end of storage period (25 and 35days, respectively).

5.3.7. Effect of refrigerated storage on sensory quality attributes of smoked Mullet fish

Organoleptic properties of fishery products are correlated significantly with their chemical, physical and microbiological characteristics. Cold and hot smoked products processed from Mullet fish obtained from the two farms (A) and (B) were evaluated organoleptically at intervals of 5 days during refrigerated storage at 3±1°C to assess the storage stability of such products. The panelists were asked to evaluate the sensory properties of the product in terms of color, taste, odor, texture and

overall acceptability. The collected data from the evaluation were statistically analyzed.

5.3.7.1. Color

The results indicated that the color of the smoked Mullet fish samples obtained from farm (A) was highly accepted by the panelists as indicated from the high values scored for cold smoked (8.0) and hot smoked samples (9.0) immediately after smoking (zero time of storage). These values were significantly $P<0.05\%$ decreased during storage to values of 4.5 and 5.0 after storage periods of 20 and 35 days, respectively.

Also, color of cold and hot smoked Mullet fish obtained from farm (B) was highly accepted by the panelists as shown from the high score values recorded by 8.5 and 9.2, respectively immediately after smoking. Meanwhile, during cold storage these values significantly ($P<0.05\%$) decreased to 5.5 and 6.2 at the end (25) and (35) days of storage, respectively.

5.3.7.2. Taste

show the changes in taste of smoked Mullet fish samples obtained from farms (A) and (B) during refrigerated storage at $3\pm1^{\circ}\text{C}$. At zero time of storage, taste of cold and hot smoked samples from farm (A) scored the values of (7.5) and (8.5); respectively. As the storage period prolonged taste values gradually decreased. At the end of 20 and 35 days of storage these values significantly ($P<0.05\%$) decreased to 5.0 and 5.5, respectively. In comparison with samples from farm (A), the results indicated that taste score values for cold and hot smoked samples from farm (B) were as high as 8.0 and 9.5 at zero time of storage indicating the good acceptance of smoked samples from farm (B). Meanwhile, as the storage time extended score values for taste gradually decreased ($P<0.05\%$) to 5.2 and 6.0 at the end of 25 and 35days storage, respectively.

5.3.7.3. Odor

Data showed that odor of the smoked Mullet fish samples obtained from farm (A) was highly accepted by the panelists as indicated from the high values. The initial odor values scored for cold smoked and hot smoked samples were as high as (8.0) and (9.0), respectively. These values significantly ($P<0.05\%$) decreased to 4.0 and 5.0 after storage periods of 20 and 35 days, respectively. Also, odor of cold and hot smoked Mullet fish obtained from farm (B) was highly accepted at zero time of storage as indicated from the high values recorded by 8.0 and 9.2; respectively. However, during storage these values decreased down up to 4.6 and 5.5 at the end of 25 and 35 days storage, respectively.

5.3.7.4. Texture

The initial values for cold and hot smoked samples from farm (A) were of 7.5 and 8.6, respectively. These values gradually decreased to 5.0 and 6.0 after 20 and 35 days of storage, respectively. Also, texture of cold and hot smoked Mullet fish obtained from farm (B) was highly accepted as the recorded values were 8.5 and 9.0; respectively at zero time of storage. These values significantly ($P<0.05\%$) decreased to 5.5 and 6.0 at the end of 25 and 35 days storage, respectively.

5.3.7.5. Overall acceptability

The initial score values of the overall acceptability of cold and hot smoked Mullet fish samples obtained from farm (A) were 7.8 and 8.8, respectively indicating the good qualities and high acceptance of the smoked Mullet fish. During storage, these values were significantly ($P<0.05\%$) decreased to 4.6 and 5.4, respectively at the end of 20-35 days of refrigerated storage. Also, overall acceptability of cold and hot smoked Mullet fish obtained from farm (B) was highly accepted but the score values were decreased from 8.4 and 9.2 at zero time of storage to 5.2 and 5.9 at the end of 25 and 35 days storage, respectively.