



Evaluation of some quality indices in farmed fish from Kafr Elshiekh governorate

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ABSTRACT

Evaluation of some quality indices was carried out to determine the nutritional value of the most cultured freshwater fishes and to estimate the tolerance of these items to remain in good condition against damage. Quality indices were evaluated in 90 samples from three different fish species (*Mugil cephalus*, *Oreochromis niloticus* and *Clarias gariepinus*) 30 of each were collected at different times from different farms in Kafr Elshiekh Governorate, Egypt. Results of pH, total volatile nitrogen (TVN) "mg N /100g", trimethylamine (TMA)" mg /100g", thiobarbituric acid (TBA)" mg/kg" and peroxide value (PV)" meqO₂/kg" were 6.27 ± 0.01 , 6.84 ± 0.52 , 2.26 ± 0.19 , 1.17 ± 0.08 and 2.31 ± 0.27 for *Mugil cephalus* 6.34 ± 0.01 , 8.97 ± 0.69 , 3.54 ± 0.26 , 1.62 ± 0.15 and 3.16 ± 0.22 in *Oreochromis niloticus* and 6.46 ± 0.01 , 12.31 ± 1.14 , 5.70 ± 0.43 , 2.89 ± 0.32 and 4.74 ± 0.51 in *Clarias gariepinus*, respectively. This study shows superiority of *Clarias gariepinus* and *Mugil cephalus* over *Oreochromis niloticus* in their nutritional value.

Keywords: *Mugil cephalus*, *Oreochromis niloticus* and *Clarias gariepinus*, trimethylamine, thiobarbituric acid.

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1. INTRODUCTION

Fish considered as a desirable and nutritional source of protein and good supply of vitamins and minerals occupying the major part of human diet (Hastein et al., 2006). In addition, fish, regarded as one of the most perishable foods and as a result of wideness of food trade, fish products become more susceptible to rejection due to their poor quality despite the technological developments in fish production (FAO, 2009).

Freshness assurance is one of the most significant goals for the fish industry as freshness is closely related to quality. Determination of post-mortem pH provides

indications of physical properties changes occurring in fish muscle during storage period (Izumi, 2012).

Fish quality involves a wide range of factors; which important for the consumer as nutritional quality, availability and integrity, freshness, eating quality and the obvious physical properties of species, type and size (Abbas et al., 2008).

Volatile amines (TVN, trimethyl-amine) are molecules responsible for fishy odor and flavor in fish which last for several days after catching and they used as measurement for assessing the fish quality (Etienne et al., 2005).

This study was carried out for evaluation the nutritive value and quality assessment of three fish species (*Mugil cephalus*, *Oreochromis niloticus* and *Clarias gariepinus*) through measurement of general indices (pH), proteolytic indices [total volatile nitrogen (TVN), trimethylamine (TMA)] , lipolytic indices (thiobarbituric acid (TBA)) and peroxide value (PV).

Materials and methods

2.1. Collection of samples:

Ninety random samples of farmed fish of *Mugil cephalus*, *Oreochromis niloticus*, and *Clarias gariepinus* (30 of each) were collected from different fish farms located in Kafr Elshiekh Governorate, Egypt. The weight of samples ranged from 200 g (*Mugil cephalus* & *Oreochromis niloticus*) to 400 g (*Clarias gariepinus*). Each sample was kept in a separated plastic bag and preserved in an ice box then transferred to the laboratory (Central laboratory for diagnosis and research of diseases of fish, faculty of veterinary medicine in Kafrelshiekh governorate) and examined chemically as quickly as possible to determine their keeping and safety for human consumption.

2.2. Quality indices:

2.2.1. Determination of pH:

pH of fish samples was determined according to Pearson (2006). Briefly in a blender, approximately 10 g of the sample were blended in 10ml of neutralized distilled water. The homogenate was kept at room temperature for 10 minutes with shaking. The pH value was measured by using an electrical pH meter (Bye Model 6020, USA). Calibration of pH meter was done by using two buffer solutions of exactly known pH (alkaline pH 7.01, acidic pH 4.01). Therefore, pH electrode was washed with neutralized water and then introduced into the homogenate.

2.2.2. Determination of Total Volatile Nitrogen (TVN):

According to FAO (1980), the technique applied as following:

In a clean dry beaker, 10g of the sample were added to 30 ml of distilled water and thoroughly mixed by a blender for 2 minutes. Thus, 2 drops of 0.02 M HCL (Al Biariq Co., SA) were added to bring pH to 5.2. The homogenate was heated to 70°C and cooled to room temperature and filtered. Accurately, the outer ring was filled with 2ml of the sample extract and 1ml of saturated potassium carbonate (KCO₃) (Shanghai Yixin Chemical Co., ltd, China). The Conway unit was rotated as gently as possible and the dish was covered and incubated at 36°C for 2 hours, HCL in inner ring was titrated against 0.01M NaOH (Richie products Co., England) by using methyl red indicator (T1 ml) (Sigma-Aldrich, Egypt). Total volatile nitrogen was estimated using the following equation:

$$\text{TVN}/100\text{g} = 26.88 \times (2 - T1)$$

Where, T1 was the volume of NaOH in the titration.

2.2.3. Determination of Trimethylamine (TMA):

The method recommended by FAO (1980) was applied using Conway test. Actually, 2ml of 0.05 M H₂SO₄ (Shijizhuang Xinlongwei chemical Co., ltd, China) were added into the inner compartment of Conway dish, however, the outer ring of the dish was filled with 2 ml of the sample extract and 1ml of saturated potassium carbonate (KCO₃) (Shanghai Yixin Chemical Co., ltd, China). Furthermore, the dish was covered and incubated at 36°C for 2 hours. Thus, H₂SO₄ in the inner ring was titrated against 0.01M NaOH by using methyl red indicator (T2 ml).

$$\text{TMA}/100\text{g} = 26.88 \times (2 - T2)$$

Where,

T2 = volume of NaOH consumed in the titration.

2.2.4. Determination of Thiobarbituric Acid (TBA):

The method used for estimation of TBA was applied as mentioned by Pikul *et al.*, (1989). TBA test depends on determination of malonaldehyde (MD) as an end product of lipid peroxidation. Actually, 10 grams of the sample were blended with 50 ml of distilled water in warring blender for 2 minutes. The mixture was transported quantitatively into Kjeldahl flask (C-5420 flask pyrex, USA) and washed with an additional 47.5 ml of distilled water. However, 2.5 ml of HCL (Al Biariq Co., SA) were added to 125 ug/g fat and then a small amount of stones was placed to prevent pumping. The flask was heated at the extreme heat obtainable in the Kjeldahl distillation apparatus (classical macro kjeldahl, Germany). After 10 minutes of boiling, 50 ml of the distillate was collected. Therefore, 5 ml of the distillate were mixed to 5 ml of TBA reagent in 50 ml glass stoppered tube. The contents were mixed and put in a boiling water bath for 35 minutes. Heating, cooling under tap water for 10 minutes was applied. The optical density of sample for the blank was measured by using Spectrophotometer (UNICAM969AA Spectronic, USA) at a wavelength 538 nm.

TBA value = $R \times 7.8$ (mg malonaldehyde /Kg)

Where R = Reading of sample against blank

2.2.5. Determination of Peroxide value:

Peroxide value was estimated according to the method carried by Asakawa and Matsushita (1978). Briefly, 3g of the sample were mixed with 10 ml chloroform into a 250 ml Erlenmeyer flask. Furthermore, 15 ml acetic acid and 1.0 ml KI solutions were added and mixed. The mixture was left for 5 minutes in a dark place. Thus, 30 ml distilled water and 1 ml starch indicator was added. The mixture was titrated with sodium thiosulfate until blue color disappeared. The titration procedure was repeated at least 3 times and the individual results shouldn't vary more than 0.3 ml.

2.3. Statistical analysis:

The obtained results were statistically evaluated by application of Analysis of

Variance (ANOVA) test according to Feldman *et al.* (2003).

3. RESULTS

pH values in the examined fish samples were varied from 6.16 to 6.39 with an average of 6.27 ± 0.01 in *M. cephalus*. While in *O. niloticus* was ranged from 6.21 to 6.53 with an average of 6.34 ± 0.01 . In *C. gariepinus*, it ranged from 6.28 to 6.67 with an average of 6.46 ± 0.01 (Table 1). The differences between species were non-significant. 94.44% of samples were accepted while 5.56% were not accepted as shown in table (2).

Total Volatile Nitrogen (TVN) values in examined fish samples were varied from 3.78 to 9.41 mg N/100g with an average of 6.84 ± 0.52 mg N/100g in *M. cephalus*. In *O. niloticus* was from 5.17 to 13.09 mg N/100g with an average of 8.97 ± 0.69 mg N/100g and from 6.85 to 18.32 mg N/100g with an average of 12.31 ± 1.14 mg N/100g in *C. gariepinus* as in (Table 3). There were high significant differences among examined samples ($P < 0.01$). All examined samples were accepted as shown in table (4).

Trimethylamine (TMA) values in the examined fish samples showed range from 1.06 to 3.35 mg /100g with an average of 2.26 ± 0.19 mg /100g in *M. cephalus*. However, in *O. niloticus* revealed TMA value varied from 1.92 to 4.67 mg /100g with an average of 3.54 ± 0.26 mg /100g. *C. gariepinus* recorded TMA value ranged from 3.18 to 7.41 mg /100g with an average of 5.70 ± 0.43 mg /100g (Table 5). The differences between samples were significant ($P < 0.05$). All the examined samples were accepted as shown in table (6). Thiobarbituric acid (TBA) values were varied from 2.09 to 3.65 mg /kg with an average of 2.89 ± 0.32 in *Mugil cephalus* and from 0.87 to 1.51 mg /kg with an average of 1.17 ± 0.08 mg /kg in *O. niloticus*. Meanwhile, *C. gariepinus* recorded TBA value from 1.14 to 1.93 mg /kg with an average of 1.62 ± 0.15 mg

Evaluation of some quality indices in farmed fish from Kafr Elshiekh governorate

/kg (Table 7). The differences among examined fish were highly significant ($P < 0.01$). All the examined samples were accepted as shown in table (8).

Peroxide value (PV) values in the examined samples were presented in table (9). *M.cephalus* showed PV value varied from 1.49 to 3.05 meqO₂/kg with an average of $2.31 \pm$

0.27 meqO₂/kg. While, in *Oniloticus* was from 2.24 to 3.81 meqO₂/kg with an average of 3.16 ± 0.22 meqO₂/kg, PV value was from 3.67 to 5.93 meqO₂/kg with an average of 4.74 ± 0.51 meqO₂/kg in *C.gariepinus*. Differences between fish were high significant ($P < 0.01$).

Table1: pH value in the examined fish samples (n=30)

Fish species	Min	Max	Mean \pm S.E
<i>Mugil cephalus</i>	6.16	6.39	6.27 ± 0.01
<i>Oreochromis niloticus</i>	6.21	6.53	6.34 ± 0.01
<i>Clarias gariepinus</i>	6.28	6.67	6.46 ± 0.01

NS = Non-significant differences

Table 2: Acceptability of the examined farmed fishes according to their pH values (n=30)

Fish species	Maximum Permissible Limit *	Accepted samples		Unaccepted samples	
		No.	%	No.	%
<i>Mugil cephalus</i>	6.5	30	100	0	0
<i>Oreochromis niloticus</i>	6.5	29	96.7	1	3.3
<i>Clarias gariepinus</i>	6.5	26	86.7	4	13.3
Total (90)		85	94.44	5	5.56

* Egyptian Organization for Standardization "EOS" (2005).

Table 3: Total Volatile Nitrogen (TVN) "mg N/100g" in the examined fish samples (n=30)

Fish species	Min	Max	Mean \pm S.E
<i>Mugil cephalus</i>	3.78	9.41	6.84 ± 0.52
<i>Oreochromis niloticus</i>	5.17	13.09	8.97 ± 0.69
<i>Clarias gariepinus</i>	6.85	18.32	12.31 ± 1.14

++ = High significant differences ($P < 0.01$)

Table 4: Acceptability of the examined farm fishes according to their TVN values (n=30)

Fish species	Maximum Permissible Limit (mg%)*	Accepted samples		Unaccepted samples	
		No.	%	No.	%
<i>Mugil cephalus</i>	30	30	100	0	0
<i>Oreochromis niloticus</i>	30	30	100	0	0
<i>Clarias gariepinus</i>	30	30	100	0	0
Total (90)		90	100	0	0

* Egyptian Organization for Standardization "EOS" (2005).

Table 5: Statistical analytical results of Trimethylamine (TMA) "mg/100g" in the examined samples of farm fishes (n=30)

Fish species	Min	Max	Mean ± S.E
<i>Mugil cephalus</i>	1.06	3.35	2.26 ± 0.19
<i>Oreochromis niloticus</i>	1.92	4.67	3.54 ± 0.26
<i>Clarias gariepinus</i>	3.18	7.41	5.70 ± 0.43

+ = Significant differences (P<0.05)

Table 6: Acceptability of the examined farmed fishes according to their TMA values (n=30)

Fish species	Maximum Permissible Limit (mg%)*	Accepted samples		Unaccepted samples	
		No.	%	No.	%
<i>Mugil cephalus</i>	10	30	100	0	0
<i>Oreochromis niloticus</i>	10	30	100	0	0
<i>Clarias gariepinus</i>	10	30	100	0	0
Total (90)		90	100	0	0

* Egyptian Organization for Standardization "EOS" (2005)

Table 7: Thiobarbituric acid (TBA) "mg/Kg" in the examined fish samples (n=30)

Fish species	Min	Max	Mean ± S.E
<i>Mugil cephalus</i>	2.09	3.65	2.89 ± 0.32
<i>Oreochromis niloticus</i>	0.87	1.51	1.17 ± 0.08
<i>Clarias gariepinus</i>	1.14	1.93	1.62 ± 0.15

++ = High significant differences (P<0.01)

Table 8: Acceptability of the examined farmed fish according to their TBA values

Fish species	Maximum Permissible Limit (mg/Kg)*	Accepted samples		Unaccepted samples	
		No.	%	No.	%
<i>Mugil cephalus</i>	10	30	100	0	0
<i>Oreochromis niloticus</i>	10	30	100	0	0
<i>Clarias gariepinus</i>	10	30	100	0	0
Total (90)		90	100	0	0

* Egyptian Organization for Standardization "EOS" (2005)

Table 9: Peroxide value (PV) "meqO2/kg" in the examined fish samples (n=30)

Fish species	Min	Max	Mean ± S.E
<i>Mugil cephalus</i>	1.49	3.05	2.31 ± 0.27
<i>Oreochromis niloticus</i>	2.24	3.81	3.16 ± 0.22
<i>Clarias gariepinus</i>	3.67	5.93	4.74 ± 0.51

++ = High significant differences (P<0.01)

4. DISCUSSION

Chemical indices such as (pH, TVN, TMA, TBA and PV) are the most widely used parameters to evaluate fish quality (El-Marrakchi et al., 1990). In this study, three type of farmed fish (*M. cephalus*, *O. niloticus* and *C. gariepinus*) were examined chemically to determine their keeping and safety for human consumption.

Concerning pH, the obtained results were nearly closed to those of Ibrahim (2017) who examined *M. cephalus* and *O. niloticus* and lower than those obtained by Sathivel (2005). pH values was a suitable index for freshness assessment and it can be useful as a guideline for quality of fish (Ruiz Capillas and Moral, 2001). The differences between species were non-significant. Egyptian Organization for Standardization (EOS, 2005) had reported the critical limits of pH for chilled fish portion which should not be more than 6.5. Therefore, 94.44% of samples were accepted while 5.56% were not accepted with 100% accepted *C. gariepinus*.

Regarding to TVN, the obtained results were higher than those of Ibrahim (2017) who recorded TVN in *M. cephalus* with an average on f 5.65±0.39 and 7.09±0.54 in *O. niloticus* and nearly balanced with those of Mahmoud (1990). Generally, TVN is synthesized by reaction from protein mainly as a result of microbial activity (Gamal et al., 2011). Thus, TVN levels are affected by the method of catch, post mortem treatment and storage temperature as well as it differ according to fish species (Nazemroaya et al., 2011). There were high significant differences among examined samples (P<0.01). According to EOS (2005) 100% of the examined fish samples were accepted as their values are lower than the maximum permissible limits.

TMA regarded as a good index of quality for many fish species by Baixas-Nogueras et al. (2002). These results were nearly similar to those of Mahmoud (1990) and Ibrahim (2017). Huss, (1995) reported that TMA differs according to species and also it is affected by storage time and catching season. The

differences between samples were significant ($P < 0.05$). According to EOS (2005), the permissible limit of TMA was 10. Hence, all the examined samples were accepted.

These results were nearly similar to those of Ibrahim (2017), higher than those obtained by Gulsun *et al.* (2009) and Nazemroaya *et al.* (2011) and lower than that obtained by Vafakhah *et al.* (2014).

TBA is a significant quality index for fatty fish (Lynch and Frci, 1993). The higher lipids content of fish the higher TBA value in fish (Caponia *et al.*, 2004). The TBA factor is responsible for a rancid flavor, off odor, color as well as texture deterioration (Olafsdottir *et al.*, 1997). The differences among examined fish were highly significant ($P < 0.01$). According to Egyptian Organization for Standardization EOS (2005), the permissible limit of TBA was 10. Subsequently, all the examined samples were accepted

Peroxide value is a good indicator of fat oxidation (rancidity) (Adeyemi *et al.*, 2013). The results of peroxide value (PV) were lower than those obtained by Famurewa *et al.* (2017) who recorded Peroxide value in *C. gariepinus* ranged from 4.60 to 7.37 meq O₂/kg. Peroxide value is a primary indicator of fat oxidation (rancidity) (Adeyemi *et al.*, 2013). When peroxide value exceeds 10-20 meq O₂/Kg, rancid taste and smell occur (Connell, 1995). Therefore, it can be concluded that the values from this study are within the acceptable limits of spoilage. Differences between fish were high significant ($P < 0.01$).

In conclusion, the present study indicated that chemical indices as (pH, TVN, TMA, TBA and PV) are the most effective indicators for quality, freshness as well as nutritive value of fish. TVN and TMA appear as reliable indicators for determination of proteolytic activity, however, TBA is a good index for demonstration of fat rancidity. In contrast, pH

is a poor indicator of fish quality as it is widely range of variation among fish species. This study shows superiority of *C. gariepinus* over *M.cephalus* and *O. niloticus* except in TBA which was higher in *M. cephalus* due to its high lipid value than other two species. *C. Gariepinus* and *M. cephalus* considered more valuable than *O. niloticus*. Therefore, it was recommend that chemical assessment of fish samples as a rapid, accurate method and gives good indication of fish quality should be done.

5. CONCLUSION

Meat selected samples are considered a good medium for the growth of salmonella and the production of toxins. The lowest contamination was in *M.cephalus* flesh meat but the highest contamination was in minced beef the presence of this bacterial species in such samples is a result of contamination along production lines include slaughtering, preparation, distribution, storage, packaging, and sale of the products. This subsequently contributes to health risks to the consumer. So, these samples need carefully control to protect consumers against food poisoning.

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