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Chemical quality of some freshwater fish in Egyptian markets

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ABSTRACT

Total ninety random meat samples of fresh fish of *Nile tilapia*, *Bagrus bayad* and *Clarias gariepinus* (thirty of each) were collected from different Egyptian fish markets to be examined for their chemical quality indices to determine their fitness for human consumption. The results showed that the pH value was (6.32 ± 0.01) , (6.38 ± 0.02) and (6.29 ± 0.02) , TVB-N (mg/100) was (14.54 ± 0.51) , (12.71 ± 0.43) and (16.76 ± 0.46) , TBA (mg MDA/kg) was (0.82 ± 0.05) , (0.57 ± 0.03) and (0.91 ± 0.06) and TMA (mg/100) was (6.19 ± 0.23) , (5.49 ± 0.21) and (6.75 ± 0.19) in the examined samples of *Nile tilapia*, *Bagrus bayad* and *Clarias gariepinus*, respectively. So, it was appeared that the *Clarias gariepinus* samples were higher in their pH, TVB-N, TMA, TBA values than other examined samples. All examined samples were fit for human consumption except one *Clarias gariepinus* sample (3.33%) was unfit due to increasing its pH value than the limit of acceptability. This study shows superiority of *Bagrus bayad* were more than *Nile tilapia* followed by *Clarias gariepinus* samples in their chemical quality indices. So, we recommend proper handling of fish from catching till eating to preserve their human consumption fitness

1. INTRODUCTION

Fish being from the most important foods due to its nutritional qualities, where it is a good source of high-quality protein those containing essential amino acids that required for good nutrition. Also, fish provides a good source of minerals and vitamins (Onyia *et al.* 2013).

Fish is one of the perishable foods. So, because of globalization of trade of food, fish products tend to rejection because of poor quality (Huss *et al.*, 2004). For this reason, the fish and fishery products quality become of a high concern in fish industry. (Huss *et al.*, 2003).

The different methods frequently used for fish spoilage assessment were classified into 2 types: sensory methods and instrumental (physical, microbiological and biochemical) methods (Huss, 1995).

The chemical quality indices more used to examine fish quality deterioration consist of TMA and TVB-N (Howgate, 2009). Breakdown of fish protein content and fat content (agent of off-flavor and rancidity) leads to quick spoilage of fish (Daramola *et al.*, 2007).

pH was an indicator of the freshness of fish because it starts with a low value at the storage early stage that means the nutritional condition was even now good and then increases in its value when the fish storage continues. (Abbas *et al.*, 2008). This increase is caused by accumulation of alkaline material (TMA and TVB-N).

Total volatile basic nitrogen is an important parameter for the quality determination in seafood products and it is the most popular chemical indicator for spoilage of fish. Also,

it has a close relationship with bacterial counts and sensory score (Amegovu *et al.*, 2012). Total volatile basic nitrogen consists of volatile amines as, dimethylamine, trimethylamine and ammonia that produced by spoilage bacteria. And the total volatile basic nitrogen produced by autolytic enzymes during frozen storage of fish (Teklemariam, 2015). So, it is an indicator of bacterial spoilage and its increasing than permissible limit makes the fish unsafe for human consumption.

Trimethylamine oxide being from the components of non-protein nitrogen (NPN) that used for assessing of fish shelf life and freshness. (Huss and Larsen, 1980). TMA was formed by post-mortem bacterial degradation of trimethylamine oxide (TMAO). Volatile amines accumulation plays a higher role in the fish products quality loss due to the unpleasant odors that is combined with the products degradation. Increasing of the TMA content leads to increasing in the TVB-N content during spoilage (Jinadasa, 2014).

Fish lipids contain very high polyunsaturated fatty acids amount that are susceptible to oxidative rancidity. During the fish storage, fish lipid oxidation and hydrolysis occurred. This leads to prevention of the fish acceptance for human consumption as a result of influencing rancidity development, texture changes, and protein denaturation (Verma *et al.* 1995). Lipid oxidation is a major cause of many problems that decrease the fish product shelf-life. It leads to texture and color changes as well as rancid odor and off-flavor. Thiobarbituric acid test based on the reaction of TBA reagent with

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malonaldehyde for obtaining a red color, that formed due to the condensation of 2TBA molecules with a molecule of malonaldehyde and the likely elimination of 2 molecules of water (Fernandez *et al.*, 1997). And it is indication for oxidative rancidity. Malondialdehyde is the lipid oxidation major product have carcinogenic and genotoxic effect (Hartman, 1983).

The maximum permissible limit of TVB-N, TBA, TMA, pH value in fresh and chilled fish was 30 mg/100g, 4.5 mg MAD/kg, 10mg/100g and 6.5 (E.S., 2005). Bacterial contamination during handling of fish make breakdown of fish tissue that leads to increasing in the values of chemical quality indicators than its permissible limits. So, fish become unfit for human consumption.

The present study aimed to determine the fitness of some freshwater fish in Egyptian markets for human consumption based on their chemical quality indices

2. MATERIAL AND METHODS

2.1. Samples

2.1.1. Samples collection:

Ninety random meat samples of fresh fish of *Nile tilapia*, *Bagrus bayad* and *Clarias gariepinus* (30 of each) were collected from different Egyptian fish markets located in El- Kalubia, Cairo and Giza governorates. Each sample was kept and transferred to the laboratory as quickly as possible in an insulated ice box. And then, the collected samples subjected to the chemical examinations to evaluate the fish quality indices of the fish species.

2.2. Chemical quality indices:

2.2.1. pH (E.S., 2017).

10 g of homogenized fresh water fish samples was added in 100 ml of pre boiled cooled distilled water at 25°C and checked for 30 min and was leaved on aside for 10 min then, apart from aqueous layer was added to a beaker and pH was measured by pH meter that was previously calibrated using standard buffers of pH 4 and 7 at room temperature.

2.2.2. Total volatile basic nitrogen (TVB-N) determination (E.S., 2017).

Nearly 10 gram of previously prepared and homogenized fish sample was put on a conical flask with 300 mL of D.W. Then, 2 g of magnesium oxide was added followed by antifoaming agent as some glass beads. The distillate was received in Erlenmeyer flask containing 25 mL of boric acid solution (2%) until the final distillate reach 125 mL in its volume. Titration of the distillate was occurred by using H₂SO₄ solution (0.1N) till the natural red end point. The same procedures were occurred by using D.W instead of the samples for blank detection. TVB-N (mg N/100g) of fish flesh was calculated as follows:

$$\text{TVB-N (mg N/100g)} = (S - B) \times 14$$

Where: S = H₂SO₄ volume that was used for sample titration, and B = volume of H₂SO₄, that was titrated for blank

2.2.3. Trimethylamine (TMA) determination (E.S., 2017).

200 ml of TCA solution (7.5%) was added to 100 g of minced fish meat then mixed well and centrifuged at 3000 rpm till the clear supernatant was obtained. 1 ml of sample supernatant and 3 mL of D.W was added to a test tube. On another 3 test tubes 1 ml, 2 ml and 3 ml of TMA standard solution was added and completed to 4 ml by D.W. On the

blank test tube, we added 4 ml of D.W. In all test tubes 1ml formaldehyde solution (20%), 10 mL toluene and 3 mL potassium carbonate solution were added then the test tubes were shaken vigorously. 8 mL of the toluene layer was put on 0.1 ml anhydrous sodium sulfate then shaken for toluene dehydration. On a test tube 5 ml from the dehydrated toluene layer was mixed with 5 mL of picric acid solution. After that, the optical density was measured photometrically by spectrophotometer at 410 nm wavelength.

$$\text{Calculation: TMA (mg /100g)} = D \times \text{Wt.} \times V \times 300 / A$$

Where: D = sample absorbance, Wt. = mg of TMA-N/ml standard solution, V = ml of used standard solution; 300 = approximation total supernatant (ml) (200 ml + 100 g), and A =standard absorbance nearest to sample absorbance.

2.2.4. Thiobarbituric acid (TBA) determination (E.S., 2017).

Approximately, ten grams of prepared fish sample was mixed with distilled water, HCl solution (4N) and Anti-pumping stones in a distilling flask. The distillation was done till collection of 50 ml distillate on an empty flask. 5 ml distillate was mixed with 5 ml TBA reagent in attest tube that was put for 35 min into boiling water bath. The blank was done by using 5 ml D.W instead of sample with the 5 ml of TBA reagent. The sample optical density (D) was read at a wavelength of 538 nm against the blank on the spectrophotometer.

$$\text{Calculation: TBA (mg MDA /kg)} = D \times 7.8$$

3. RESULTS

From the results presented in table (1),it was appeared that the pH value of the examined *Nile tilapia*, *Bagrus bayad* and *Clarias gariepinus* samples were varied from (6.15 to 6.32, 6.08 to 6.48 and 6.22 to 6.67), respectively.

Table 1 Statistical analysis of pH values in the examined freshwater fish samples (n=30).

Fish samples	<i>Nile tilapia</i>	<i>Clarias gariepinus</i>	<i>Bagrus bayad</i>
Min.	6.15	6.22	6.08
Max.	6.49	6.67	6.48
Mean ± SE.	6.32±0.01 ^A	6.38±0.02 ^B	6.29±0.02 ^A
Max. Per. Limit	6.5	6.5	6.5
Accepted samples	NO.	30	29
	%	100	96.67
			30
			100

There are sig. diff. (P<0.05) between means having the different capital letters. * According to E.S (2005). F value = 7.55

Results presented in table (2) revealed that the TVB-N value of the examined *Nile tilapia*, *Bagrus bayad* and *Clarias gariepinus* samples were varied from (7.28 to 18.48, 8.68 to 17.92 and 11.76 to 21.28) mg N/100g with an average of (14.54± 0.51, 12.71± 0.43 and 16.76± 0.46) mg N/100g, respectively.

Table 2 Statistical analysis of TVB-N (mg/100g) in the examined samples of freshwater fish (n=30).

Fish samples	<i>Nile tilapia</i>	<i>Clarias gariepinus</i>	<i>Bagrus bayad</i>
Min.	7.28	11.76	8.68
Max.	18.48	21.28	17.92
Mean ± SE.	14.54 ± 0.51 ^A	16.76 ± 0.46 ^B	12.71 ± 0.43 ^C
Max. Per. Limit (mg/100g)*	30	30	30
Accepted samples	NO.	30	30
	%	100	100
			30
			100

There are sig. diff. (P<0.05) between means having the different capital letters. * According to E.S. (2005). F value = 18.846

Table (3) showed that the TMA (mg N/100g) value of the examined samples was varied from (2.69 to 8.23) with an average of 6.19 ± 0.23 in *Nile tilapia*, varied from (4.20 to 8.74) with an average of 6.75 ± 0.19 in *Clarias gariepinus* and varied from (2.52 to 7.56) with an average of 5.49 ± 0.21 in *Bagrus bayad*.

Table 3 Statistical analysis of TMA (mg/100g) in the examined fish samples of fresh water sources (n=30).

Fish samples	<i>Nile tilapia</i>	<i>Clarias gariepinus</i>	<i>Bagrus bayad</i>
Min.	2.69	4.20	2.52
Max.	8.23	8.74	7.56
Mean \pm SE.	6.19 ± 0.23^A	6.75 ± 0.19^A	5.49 ± 0.21^B
Max. Per. Limit (mg/100g)*	10	10	10
Accepted samples	NO.	30	30
	%	100	100

There are sig. diff. ($P < 0.05$) between means having the different capital letters. * According to E.S. (2005). F value = 8.615

From the results recorded in table (4) it is evident that the TBA (mg Malondialdehyde/kg) varied from 0.42 to 1.48 with an average of 0.82 ± 0.05 in examined *Nile tilapia* samples, 0.53 to 1.70 with an average of 0.91 ± 0.06 in examined *Clarias gariepinus* samples and 0.36 to 1.07 with an average of 0.57 ± 0.03 in examined *Bagrus bayad* samples.

Table 4 Statistical analysis of TBA (mg Malondialdehyde/kg) in the examined samples of freshwater fish (n=30).

Fish samples	<i>Nile tilapia</i>	<i>Clarias gariepinus</i>	<i>Bagrus bayad</i>
Min.	0.42	0.53	0.36
Max.	1.48	1.70	1.07
Mean \pm SE.	0.82 ± 0.05^A	0.91 ± 0.06^A	0.57 ± 0.03^B
Max. Per. Limit (mg MDA/kg)*	4.5	4.5	4.5
Accepted samples	NO.	30	30
	%	100	100

There are sig. diff. ($P < 0.05$) between means having the different capital letters. * According to E.S. (2005). F value = 13.152

4. DISCUSSION

Chemical quality indicators as (TBA, TMA and TVB-N) were used for evaluation of fish quality (El-Marrakchi *et al.*, 1990). In this research, 3 types of freshwater fish (*Nile tilapia*, *Bagrus bayad* and *Clarias gariepinus*) were examined for determining their indices of chemical quality and their human consumption safety.

The pH is a remarkable intrinsic parameter related to fish meat and affect the freshness as, its affection by bacterial growth (Gram and Huss, 1996). Results obtained in table (1) were appeared that the mean pH values were 6.32 ± 0.01 in *Nile tilapia* was lowered than 6.38 ± 0.02 that's of *Clarias gariepinus* and was higher than 6.29 ± 0.02 that's of *Bagrus bayad*. This results was nearly similar to results recorded by Alparslan *et al.* (2013) (6.35 ± 0.02) in raw *sea bass*, Ghannam *et al.* (2015) (6.32 ± 0.01 and 6.25 ± 0.02) in *catfish* during winter and spring seasons, respectively and Gonçalves *et al.* (2018) reported that the initial pH of the fish sample was 6.4. Higher result obtained by Khidhir *et al.* (2013) (from 6.757 to 7.908), Ghannam *et al.* (2015) (from 6.85 ± 0.01 to 7.11 ± 0.02) in *Nile tilapia* and Gerges, *et al.*, (2016) (6.5 ± 0.02) in control *Nile tilapia* fillet at 0 day of examination. Lower result obtained by Gamal El-Deen and El-Shamery (2010) (from 5.3 to 5.7) and Alparslan *et al.* (2013) (4.32 ± 0.04) in scaly Marinated *sea bass*. There were significant differences among examined (*Nile tilapia* and *Clarias gariepinus*) and (*Clarias gariepinus* and *Bagrus bayad*) samples ($P < 0.05$). Differences of pH values were affected by differences in the diet, species, seasons, type of muscle and the stress level during fish catching or due to different bacterial contamination levels which lead to formation of different levels of alkaline metabolites as TVB-N and TMA. There were no significant differences ($P > 0.05$) among examined (*Nile tilapia* and *Bagrus bayad*) samples. All examined samples were accepted in pH value according to "E.S" (2005) except one *Clarias gariepinus* sample (3.33%) was unaccepted. TVB-N being, a perfect indicator for determination of the freshness and quality measurement in *Nile Tilapia* and *African catfish* (Liu *et al.*, 2010). The results recorded in table (2) was appeared that the TVB-N value (mg N/100g) of the examined samples of *Nile tilapia* (14.54 ± 0.51) was lower than that of *Clarias gariepinus* (16.76 ± 0.46) and was higher than that of *Bagrus bayad* (12.71 ± 0.43). This result was nearly similar to results obtained by Alparslan *et al.*, (2013) (16.89 ± 1.45 mg/100g) in raw *sea bass*, Ghannam *et al.*, (2015) (16.01 ± 0.14 mg/100g in *Nile tilapia* during winter season) and Hassan *et al.*, (2019) who recorded TVB-N was 12.31 ± 1.14 mg /100g in *Clarias gariepinus* samples. Higher result obtained by Jianadasa *et al.*, (2014) (52 in mg/100g *sailfish*), Ghannam *et al.*, (2015) (19.27 ± 0.19 mg/100g in *catfish* during summer season) and Enenwa *et al.*, (2019) (21.95 to 55.44 mg/100g in *Trachurus trachurus* samples). Lower result obtained by, Alparslan *et al.*, (2013) (9.82 ± 0.87 mg/100g in scaly Marinated *sea bass*), Enenwa *et al.*, (2019) (8.78 to 9.47) mg /100g in *Clupea harengus*, and Hassan *et al.*, (2019) (6.84 ± 0.52 and 8.97 ± 0.69) mg/100g in *M. cephalus* and *O. niloticus* samples, respectively. There were significant differences among different species of examined samples ($P < 0.05$). This was caused by different count of bacterial contamination from environment or improper handling and preservation that lead to protein degradation and releasing the TVB-N. TVB-N was good seafood advanced spoilage chemical indicator, but it was an insufficient as quality sign for seafood initial stages of spoilage (Tejada and Huidobro, 2002). All examined samples (100%) were accepted in TVB-N according to "E.S" (2005). Tri-methylamine originates from bacterial decomposition therefore, it was considered as an indicator for bacterial growth (Jianadasa *et al.*, 2014). It was answerable for fishy flavor and odor that was appeared after several days from fish catching (Etienne *et al.*, 2005). Results obtained in table (3) was revealed that TMA (mg/100g) content was higher in *Clarias gariepinus* than *Nile tilapia* followed by *Bagrus bayad*. Nearly similar results were recorded by Enenwa *et al.*, (2019) (6.55 mg/100g in 1st collection of *Sardina pilchardus*) and Hassan *et al.*, (2019) 5.70 ± 0.43 mg/100g in *Clarias gariepinus*. Higher result reported by Dergal *et al.*, (2013) (8.49 ± 0.43 mg/100g) in refrigerated *Nile tilapia* and Enenwa *et al.*, (2019) (20.77 and 40.67) mg/100g in 1st and 2nd Collection of *Trachurus trachurus* samples, respectively. Lower result obtained by Alparslan *et al.*, (2013) (ranged from 2.86 ± 0.1 to 3.21 ± 0.12) mg/100g, Dergal *et al.*, (2013) (0.65 ± 0.16 mg/100g) in fresh *Nile tilapia*, Ghannam *et al.*, (2015) (varied from 0.45 ± 0.02 to 0.95 ± 0.05) mg/100g, Enenwa *et al.*, (2019) (3.37 and 1.39) mg/100g in 2nd and 3rd Collection of *Sardina pilchardus*, and Hassan *et al.*, (2019) (2.26 ± 0.19 mg/100g for *M. cephalus* and 3.54 ± 0.26 mg/100g for *O. niloticus*) samples. There were significant differences ($P < 0.05$) among examined (*Nile tilapia* and *Bagrus bayad*) samples and between the examined (*Clarias gariepinus* and

Bagrus bayad) samples. TVB-N and TMA were affected by the activity of spoilage bacteria and the effect of endogenous enzymes (Kilinc and Cakli, 2004). There were no significant differences ($P>0.05$) among examined (*Nile tilapia* and *clarias gariepinus*) samples. All examined samples were accepted in TMA according to "E.S" (2005). The TBA value was widely used in fish and fish products for measuring lipid oxidation (Yanar et al., 2006). And it was responsible for off odor, rancid flavor, texture as well as color deterioration (Olafsdottir et al., 1997). It is evident from the results recorded in table (4) that the TBA values (mg Malondialdehyde/kg) were lower in *Bagrus bayad* than *Nile tilapia* than *Clarias gariepinus* examined samples. The TBA results in the examined samples were nearly similar to those obtained by Ghannam et al., (2015) (from 0.50 ± 0.05 to 0.80 ± 0.01) mg Malondialdehyde/kg in *Nile tilapia* and Enenwa et al., (2019) (0.5 and 0.9) mg Malondialdehyde/kg in 1st and 2nd Collection of *Clupea harengus*, respectively. Lower results were obtained by Alparslan et al., (2013) (0.30 ± 0.01 mg MDA/kg) in raw sea bass, Ghannam et al., (2015) (0.40 ± 0.04 and 0.45 ± 0.02) mg MDA/kg in catfish during winter and spring seasons, respectively and Enenwa et al., (2019) (0.39 mg MDA/kg) in 3rd collection of *Clupea harengus*. Higher result were reported by Alparslan et al., (2013) (7.83 ± 0.06 mg MDA/kg) in scaly Marinated sea bass, Enenwa et al., (2019) (from 2.82 to 4.00) mg MDA/kg in *Trachurus trachurus* and Hassan et al., (2019) (3.16 ± 0.22 mg MDA/kg) in *Oreochromis niloticus*. There were significant differences ($P<0.05$) among examined (*Nile tilapia* and *Bagrus bayad*) samples and between the examined (*Clarias gariepinus* and *Bagrus bayad*) samples, but There were no significant differences ($P>0.05$) among examined (*Nile tilapia* and *Clarias gariepinus*) samples. This may be explained by different handling or storage or preservation or species or environment where in the most of aquaculture ponds the *Nile tilapia* and *clarias gariepinus* were reared together. All examined samples were accepted in TBA according to "E.S" (2005).

5. CONCLUSION

There was a superiority of *Bagrus bayad* than *Nile tilapia* followed by *Clarias gariepinus* samples in their chemical quality indices. All examined fish samples were fit for human consumption according to the chemical quality. Proper handling, preservation, sailing and processing are recommended for the preservation of the fish quality.

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